1 Overview of Cancer Biology

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A new scientific truth does not triumph by convincing its opponents and making them see the light, but rather because its opponents eventually die, and a new generation grows up that is familiar with it.

Max Planck

A discovery must be, by definition, at variance with existing knowledge. During my lifetime, I made two. Both were rejected offhand by the popes of the field. Had I predicted these discoveries in my applications, and had those authorities been my judges, it is evident what their decisions would have been.

Albert Szent-Gyorgyi

Key points

- Cancer is a genetic disease characterized by the emergence of deranged versions of normal cells, born out of aberrant molecular biology.
- Cancer is the malign byproduct of an ensemble performance in which mutations in the DNA and altered gene expression are enacted against a background of conniving environmental factors such as carcinogens and chronic inflammation.
- A large number of factors are adduced to explain the genesis of cancer, including the twin pillars of incitement of primeval urges and the emancipation from normal restraining forces. Together, these produce untrammeled cell-cycle progression.
- Studies of rare familial "monogenic" cancer syndromes have had a major impact on our fundamental understanding of cell biology, but most cancers do not result from inheritance of single, potent, cancer-causing mutations.
- Instead, they are "sporadic," with cancer-causing gene mutations arising in adult somatic cells.
- Hereditary factors may, however, exert weak and subtle influences on the risk of development and subsequently the behavior of most if not all so-called "sporadic tumors," through a complex interplay between multiple, largely unknown polymorphic alleles, some of which may only be disadvantageous if the individual is exposed to particular environmental carcinogenic factors, such as tobacco smoke.
- In general, factors that cause mutations and those that increase cell replication can combine to cause cancer, which may explain the powerful role of chronic inflammation in the causality of many carcinomas.
- Cancer is a clonal disease arising by the multistep accumulation of genetic or epigenetic changes in tumor suppressor genes, oncogenes, and "caretaker" genes that favor expansion of the new clone over the old in a process akin to Darwinian evolution.
- Natural selection will favor expansion of clones with acquired characteristics advantageous to the cancer cells, often referred to as the "hallmark" features of cancer (Fig. 1.1), which have been famously distilled by Robert A. Weinberg and Douglas Hanahan as:
  (1) the capacity to proliferate irrespective of exogenous mitogens;
  (2) refractoriness to growth inhibitory signals;
  (3) resistance to apoptosis;
  (4) unrestricted proliferative potential (immortality);
  (5) capacity to recruit a vasculature (angiogenesis);
  (6) ability to invade surrounding tissue and eventually metastasize.
- Recently, the "Warburg effect," a metabolic switch towards increasing ATP production by glycolysis, along with evasion or subversion of the immune system have been championed as the seventh and eighth hallmark features, respectively.
- RB and TP53, the doyens of tumor suppressor proteins, can arrest the cell cycle or trigger apoptosis in response to assorted cellular stresses, activation of DNA damage checkpoints, or during attempted oncogenic hijacking of cell-cycle control.

(*Continued*)

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- An intriguing question is exactly how a tumor cell with DNA damage retains so many varied options in the face of TP53 activation? Thus, TP53 can mitigate cell death and inspire DNA repair but, in complete contrast, if repairs fail it might drive a cell to celibacy or suicide.
- Put simply, with respect to replication, a cell with irreparably damaged DNA has to either kick the habit or kick the bucket.
- Not surprisingly, therefore, loss of tumor suppressors is a prerequisite for tumorigenesis. Although there is some overlap, broadly speaking, cancer cells without TP53 can survive an alarming rate of mutation, whereas absence of the other archetypal tumor suppressor, RB, represents a fountain of youth for the otherwise rapidly senescing cancer cell.
- When and where, in the life history of a cancer, do the genetic changes required for metastases occur? There is no satisfactory answer to date. Natural selection does not really provide an explanation as to why a clone of cancer cells with metastatic capabilities would be selected for in the primary tumor, unless the causal mutations first and foremost also provide a growth advantage. It is possible that potential metastatic behavior is serendipitously acquired early in tumorigenesis as a byproduct of mutations promoting growth of the ancestral primary tumor (supported by some gene expression profiling studies of whole tumors). Alternatively, it may be that mutations in specific metastases-suppressing genes that do not confer a growth advantage to the primary occur at a later stage, possibly once cancer cells have begun circulating.
- Recent intriguing questions have been posed regarding the ongoing evolution of cancer cells in primary and secondary tumors. Recent findings suggest that following an initial shared origin, clones with metastatic capabilities emerge in the primary. Once ensconced within a new secondary environment, the metastatic alumni follow a parallel and distinct evolutionary path that may intriguingly begin while still in transit.
- Cancers are complex and heterogeneous, comprising a series of genetically differing populations (clones) of cancer cells. In fact, the *dramatis personae* of cancer includes the cancer cells-elect, the profligate parents, and a number of libertine relatives of dubious provenance.
- Moreover, the whole ensemble is supported by a strong supporting cast of both collaborating and insurgent noncancer cells that together constitute the cancer microenvironment.
- The cell of origin for any given cancer – be it stem cells that partially differentiate or differentiated cells that partially dedifferentiate, continues to offer opportunities for spirited debate.
- Tumors are not egalitarian societies. Rather they are in most cases oligarchies run by a malign minority of so-called cancer stem cells (CSCs). Part gang master and part queen bee, CSCs lie embedded within a large cast of bit part players. CSCs were first described in hematological malignancies, where they are strongly implicated in maintaining the malignant phenotype. More recently, CSCs have been identified in solid tumors and may be responsible for invasive behaviors, treatment resistance, and recurrence. By implication, these cancer oligarchs are the target of the original cancer-causing mutations, suggesting that in the case of a tumor the fish rots from the head.
- CSCs share many properties and molecular markers with normal stem cells, but this does not constitute proof of paternity. Under the influence of relevant mutations, including those that provoke epithelial–mesenchymal transition (EMT), normal cells can have "stemness" thrust upon them.
- This departs from the more traditional view of indefatigable clonal competition; dog eats dog, the strongest prevails with the extinction of the weakest – *aut Caesar aut nihil.*
- The cancer microenvironment, including the inflammatory milieu and the tissue stroma (connective tissue, fatty tissue, blood vessels, and lymphatics), represents an *alma mater* for cancer cells, which by encouraging EMT can help to generate CSCs and support the success of tumorigenesis.
- The greater recognition of the portentous events unfolding within the purlieus of the tumor peripheries during tumorigenesis has already yielded dividends. Thus, the stroma plays society hostess to a prohibition-free orgy of concupiscent cancer cells, egged on by a small faction of attendant immunocytes and under the averted gaze of the rest.
- Remarkably, it now transpires that cancer-contributing mutations are no longer the sole preserve of cancer cells themselves. In fact, mutations in stromal cells may allow them to more effectively mentor cancer cells towards the achievement of their six or eight hallmark milestones.
- The molecular profile of a tumor constitutes a manifesto, within which its future behavior is adumbrated and from which its weaknesses might be divined. Moreover, seminal parts of this manifesto achieve remarkably widespread circulation. Therefore, for diagnosis it may be unnecessary to directly remove tumor tissue, because cells, proteins, and nucleic acids derived from it are continually being shed into more readily accessible body fluids.
- The search for clinically useful molecular biomarkers represents one of the most promising areas of cancer research. Many biomarkers are already in routine clinical practice, where they assist in disease monitoring and in treatment selection.
- However, biomarkers have, as yet, not helped us to paint more accurate portraits of tumors. Unfortunately, in most cases they fail to unambiguously identify their subjects. There is no "Habsburg lip" for cancers.
- In fact, biomarkers have had limited impact on screening the general population for most cancers.
- Given the increasing number of therapeutics in our arsenal against cancer, great efforts are being made to find biomarkers that may help select appropriate treatments for individual patients.
- Cancers may be cured by surgery, but only if the entire tumor is accessible and no cells have spread to other sites. Modern approaches to cancer drug development are increasingly moving away from traditional chemotherapeutic agents which paralyze cell division or cause DNA damage and instead are aimed at targeting specific cancer-relevant proteins, such as oncogenic tyrosine kinases.

- Oncogene addiction, the process by which cancer cells become critically reliant on the mutant signaling molecules, offers the potential of both effective and minimally toxic agents directed against such proteins. A potential realized by pioneering therapeutic successes, such as imatinib, used to such good effect to target the abnormal BCR–ABL fusion protein in chronic myeloid leukemia.
- However, use of these agents is in most cases severely limited by acquired or, on occasion, inherent resistance of cancer cells to the treatment. It is hoped that understanding the resistance mechanisms involved will allow rational development of combinations of targeted agents in the future, though further mutations may render even these ineffective over time. One could easily be forgiven for likening these efforts to cure cancers by drug therapy with the task set before Sysiphus.
- However, we may yet keep the boulder from rolling down the hill. Knowledge is power and by exploiting the potential of treatment biomarkers we may gain an edge over cancer. Thus, we can assess whether a given cancer will respond to particular drugs as exemplified by the presence of estrogen or progesterone receptors and mutant *NEU* in breast cancer, or may conversely suggest a response to be unlikely as in the presence of *KRAS* in colorectal cancer. Armed with enough of these biomarkers there is reason to suppose that the goal of individualized medicine and tailored therapy may soon be within reach.

Introduction

> And yet there is something so amiable in the prejudices of a young mind, that one is sorry to see them give way to the reception of more general opinions.
>
> *Jane Austen*

In this chapter we give a historical overview of cancer and go on to introduce and summarize the concepts and topics to be covered in this book. Wherever possible, we emphasize new thinking, emerging views, and novel models for studying and understanding oncogenesis. Unapologetically, this chapter aims to be stimulating and thought-provoking.

Cancer has been recognized throughout recorded history and was known to the ancient Egyptians (see Appendix 1.1 – History of cancer), but it was not until the seventeenth century that the formal study of cancer (oncology) was first documented. As with much of biology, the last 50 years has witnessed spectacular progress in describing the fundamental molecular basis of cancer following the advent of molecular biology and genetics. Frustratingly, such exponential progress in describing the biology of cancer has not yet translated into an equally impressive progress in the war against most common cancers (Fig. 1.2). We can, however, claim victory in some important skirmishes. Possibly the single greatest success has been in altering the status of cancer in many cases to that of a chronic illness. Most people now live for some time with the disease rather than rapidly succumbing to it. This is in part testimony to better treatments. At the time of going to press, the overall median survival time for the commonest 20 cancers had increased from around one year in 1971 to just under 6 years by 2007. Most of this gain, however, reflects

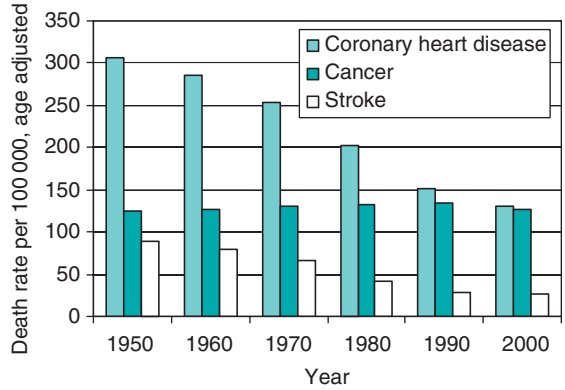


Figure 1.2 The incidence of cancer is not declining when compared to other major diseases, yet in the United States alone more than US$4.7 billion per year is spent on cancer research. Leland Hartwell and others at a meeting of the American Association of Cancer Research identified the following areas, in addition to developing new therapies, as key targets to address this major public health issue: (1) More coordinated and concerted activity between researchers. This would require establishing the necessary infrastructure for facilitating collaborative working and information exchange. (2) Testing drugs and agents in early-stage disease rather than as at present largely in end-stage cancer (we may be underestimating the potential of many drugs and therapies for this reason). (3) Real-time monitoring of treatments in early-stage cancers, though to identify earlier stages will require improved biomarkers. (4) Use of RNAi to explore combinations of targets. (5) Improved understanding of chromosomal aberration. This occurs very early in mouse tumors. (6) Exploiting genomic instability in therapeutics. Understanding more about DNA repair and repair of double-strand breaks (the latter are unusual in mouse unless telomeres are shortened). (7) Improved diagnostics from blood and body fluids – proteomics (less than 1% of proteins in blood identified, and less than 20% of these licensed for diagnostics). Data from Centers for Disease Control.

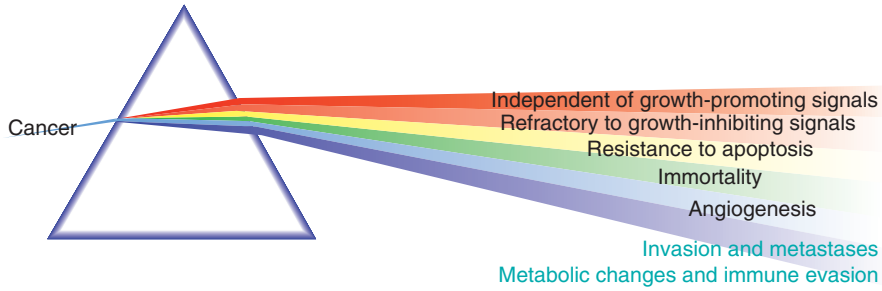


Figure 1.1 The behavior of cancers can be split into a number of common hallmark features.

the very pronounced improvements in survival from breast and colon cancers and from lymphoma. Unfortunately, over the same period, for other common cancers, notably those of lung, pancreas, and brain, improvements have been negligible and survival time is still measured in weeks.

At first glance, the biology of cancer appears straightforward. Cancer cells stop obeying the "societal" restraints imposed on individual cells within the adult organism. Instead, they multiply uncontrollably and congregate in places that should be off-limits – much like teenagers. However, in order to achieve such independence cancer cells must first be emancipated and overcome the numerous intrinsic and extrinsic barriers that seek to prevent such selfish behavior before it can threaten the survival of the entire organism.

In this book we describe the means by which normal cells are transformed into cancer cells and the key cellular processes subverted along the way. We also describe the cellular forces arraigned against the designate cancer cells and those operating on behalf of them and the weaponry available to both sides. We explain how this basic knowledge has been translated into improved diagnostics and more biologically targeted therapeutics for cancer patients. The global burden of different types of cancer is described alongside the current state of the art in diagnosis and management of cancer patients. Along the way, we make some predictions as to where new scientific and clinical breakthroughs may come from and offer our humble opinions as to why, despite some notable successes, cancer cells continue to flourish even in the face of our most sophisticated anticancer therapies. Maybe we should accept at this point that perfect theoretical proof of fact is impossible. The Münchausen Trilemma (used in philosophy to imply that it is not possible to prove any truth, even in mathematics) may provide some reassurance on this point. However, in practical terms, a good model incorporating treatments or biomarkers that work in the clinic may be a more realistic goal, even if our understanding of why the treatments work proves misguided. We are sure to continue to use the treatments until something better comes along, even if we discard the model.

Cancer poses a major threat to already overstretched healthcare services. The magnitude of the problem was summarized by Dr Gro Harlem Brundtland, former Director-General of the World Health Organization, in the Foreword to the 2003 *World Cancer Report*: "The global burden of cancer continues to increase. In the year 2000, 5.3 million men and 4.7 million women developed a malignant tumour and 6.2 million died from the disease. The number of new cases is expected to grow by 50% over the next 20 years to reach 15 million by 2020."

Cancer is responsible for more than 10% of deaths worldwide and more than 25% in some countries. Excluding the relatively frequent nonmelanoma skin cancers, lung cancer is the commonest cancer worldwide, accounting for 1.2 million new cases per year, followed closely by breast cancer and colorectal with around 1 million new cases.

The high incidence of this disease, its life-threatening nature, and often unsatisfactory management has motivated academic researchers and those from the biotechnology and pharmaceutical industries to focus on the causes and potential treatments of cancer on a scale unparalleled in almost any other disease area. Remarkably, at present there are almost 500 products in clinical trials, of which 100 are in phase III, with breast cancer and non-small-cell lung cancer receiving the most attention.

In general, cancers begin with a mutational event in a single cell and then develop in multiple stages through the acquisition of further mutations, propitious and otherwise, that are passed on to the progeny of that cell when it divides. So cancer is a clonal disease (Fig. 1.3). Aside from a few notable rare exceptions, these events arise predominantly in adult somatic cells and so are not inherited by the offspring of the affected cancer patient but only by the progeny of the affected cancer cell. In other words, transmission of the mutation ceases with the death of the patient, unless by some chance the mutant gene has been picked up by a virus, which survives and propagates. If such a virus carrying a mutated gene infects a potentially vulnerable host then the cancer-causing potential of that gene may again be unleashed upon another hapless organism. Contrary to accepted wisdom, very recent studies have suggested the astonishing possibility that cancer cells could under rare circumstances be directly inoculated from a tumor-bearing host into an unfortunate recipient. Thus, leukemias may be transmitted from mother to child and dogs may transmit cancer cells to their partners during mating.

Mutations – alterations in the coding sequence of the DNA – are not the only route to inactivation or activation of a key gene/protein. Gene expression may also be strongly influenced by a variety of **epigenetic factors** that alter chromatin structure without changing the coding DNA; these can still be passed on through successive cell generations. The term "epimutations" is often used to encompass both these major routes by which cancer cells acquire aberrant expression/activity of key genes and proteins. The average adult human has been estimated to contain as many as 10^{14} cells (i.e. 100 000 000 000 000 cells), most of which could theoretically become a cancer cell given the right sort of genetic mutations and epigenetic changes. In fact, cancer is unique in that epimutations in a single cell can give rise to a devastating disease because the resultant aberrant gene and associated antisocial behavior are transmitted to all the cellular progeny of that cell.

Because DNA replication and synthesis are essentially error prone, it is replicating cells that are most vulnerable to cancer-causing mutations. Not surprisingly, as stem cells are the main replicating cell population in the hematological system and also in epithelia, from which most cancers arise, they have long been intimated as the cell of origin for cancer. This is supported by the presence within many cancers of a side population of cells bearing stem cell characteristics known as the "cancer stem cells" (CSCs). More of this later. Although some differentiated cell types, of which adult nerve cells are a good example, are by implication unlikely to give rise to cancers because they are essentially non-proliferating in the adult, most cells either regularly replicate or can do so at a pinch. Most adult cells survive on average for 4–6 weeks and then have to be replaced. Over a hundred billion cells may die each day and are renewed either by replication of existing cells or from stem cell precursors. Given that each cell gets a substantial amount of daily DNA damage and 10^{11} or more of them will replicate each day, that is a lot of potential cancer cells!

With this in mind, a cancer might be expected to be a frequent occurrence. Yet cancer is diagnosed in only in 1 in 3 people and usually even then only after 60 or 70 years of potentially mutation-causing events. So why does a clinically apparent cancer only arise in every third individual when there are somewhere in the region of 10^{14} good potential cellular targets at risk? Moreover, we live in a world in which each of those cells is continually exposed to a myriad of avoidable and unavoidable

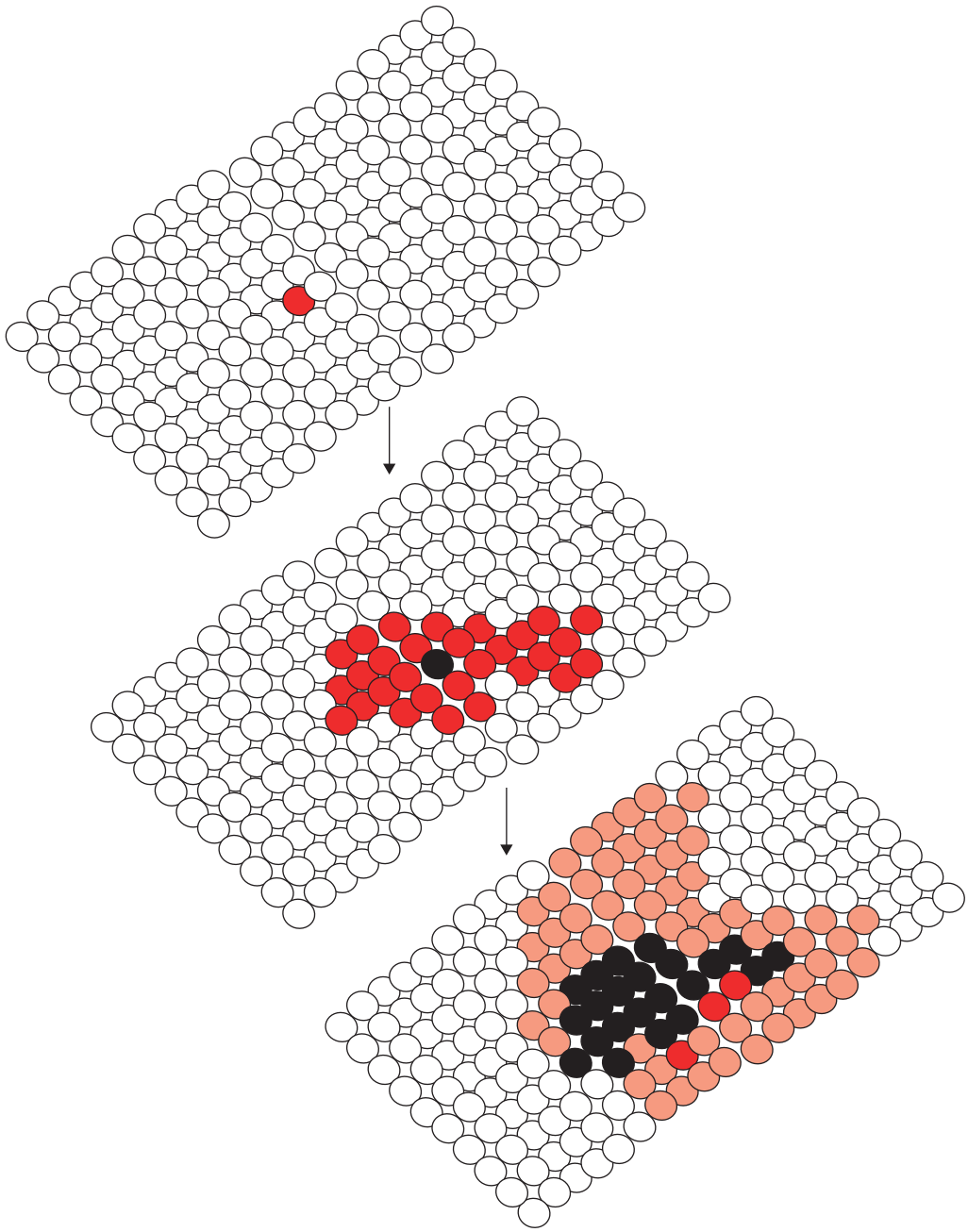


Figure 1.3 Cancer is a clonal disease. Expansion of the original clone (red) is followed by emergence of a new clone (black) which gradually replaces the original. Subsequently, a further clone (orange) emerges and expands.

DNA-damaging agents. Let us state this in the boldest terms possible. **At a cellular level, cancer is very, very rare. This surprising observation can only be accounted for by the existence of some extraordinarily effective barriers to cancer cell development. These barriers are clearly not infallible, but they must be tremendously powerful.** It is also worth reflecting on what the purpose of many of these conserved anticancer mechanisms may have originally been, given their presence in short-lived and even oligo-cellular "organisms" that are at no risk of cancer. One possibility is that processes such as senescence and apoptosis are a byproduct of archaic processes involved in balancing nutrient supply, growth and repair, and energy that fortuitously also limit cancer in longer lived multicellular organisms.

Cancers may well originate from a single bad cell, but are self-evidently not clinically detectable at that stage either by direct observation or conventional investigations such as X-ray,. This requires the presence of a small nodule, at which point replication has increased the number of cancer cells to around one billion (10^9). In other words, by the time a cancer is discovered, the original cancer cell has proceeded through some 30 or more cell divisions, and acquired a host of further epimutations. This situation is compounded by the near universal loss of normal DNA damage surveillance and repair processes. Not surprisingly,

this has complicated studies attempting to unravel the initial causes of cancer in humans. We are detectives investigating a crime that occurred some 30 generations in the past. Imagine today trying to identify the particular something, potentially quite innocuous, that happened to one of my antecedents at the time of the battle of Hastings, something which may in some unexplained way have been propagated through the ages and finally made me write this book.

Safely squirreled away within a stromal nursery, those normal cells that have been successfully emancipated will give rise to a new dynasty of proto-cancer cells. This new found freedom may result from chronic inflammation but once rendered immutable by epimutations a door to a malignant future has been forced open. The resultant unfettered cell can indulge in previously proscribed behaviors, such as unrestrained growth, and be afforded unprecedented opportunities for travel and preferment. Gradually, successive generations, honed by exposure to the hostile forces arraigned against them, will witness the emergence of increasingly malign elites that begin to dominate and supplant their forbears. If we can stretch the societal analogy further, then we may claim that normal tissues exemplify totalitarianism, whereas cancerous ones are essentially pluralist. Although with time, one or more clones may become first among equals and even have imperialist aspirations, it is now recognized that in many cases tumors continue to harbor substantial representation of earlier clonal dynasties.

A schematic of how we believe cancers arise and progress is provided in Fig. 1.4. This, can be used as an overview to be referred to while reading the more detailed (and complex) description of the basis of cancer in this book.

Cancer incidence and epidemiology

In the United Kingdom and North America, the lifetime risk of developing cancer is more than one in three, and cancer is responsible for around one in four deaths. Yet, the fear of cancer experienced by many individuals should be balanced by an appreciation that one is still far more likely to die or become disabled due to a heart attack or stroke (Fig. 1.5), if that knowledge may be in any way regarded as reassuring.

Given that almost every cell type can give rise to cancer and that more than 200 different types of cancer are recognized, it is notable that four – breast, lung, large bowel (colorectal), and prostate – account for over half of all new cases. It should also be noted that although nonmelanoma skin cancer (NMSC) is very common, with 100 000 new cases recorded each year in the United Kingdom, this data is likely incomplete and the disease usually curable, so the NMSC statistics are now routinely omitted from the overall totals. In 2006 in the United Kingdom, 293 601 people were diagnosed with cancer, excluding NMSC.

Different cancers affect people at different ages, but not surprisingly the overall risk of developing a cancer rises sharply with increasing age, with 65% of cancers in the United Kingdom occurring after the age of 65 years and 35% above age 75 (Fig. 1.6). In children, leukemia is the most common cancer (around 30% of all pediatric cancers); in young men aged 20–39 it is testicular cancer.

The incidence of cancer has changed over the last 20 years; there has been a decline in lung cancer in the United Kingdom and North America in men (but an increase in women), mainly as a result of changes in smoking habits, and an increase in breast and prostate cancer. Yet despite this, an estimated 160 000 people died from lung cancer in the United States alone in 2009. In 1981 there were 78 cases of breast cancer per 100 000 women in Great Britain, and 38 cases of prostate cancer per 100 000 men. By 2009 rates were 124 and 106, respectively (http://info.cancerresearchuk.org/cancerstats/incidence).

The International Agency for Research on Cancer (IARC) has released figures on global cancer incidence for 2008 and made predictions for the next decades (http://globocan.iarc.fr). Globally, around 12.7 million new cases and 7.6 million cancer deaths occurred in 2008, the commonest being lung (1.6 million), which makes up almost 13% of the total, breast (1.38 million), and colorectal cancers (1.23 million). The most common causes of cancer death were lung, stomach, and liver, indicating the relative success of treatments for breast and colon cancers.

It has long been appreciated that there is a geographical variation in cancer incidence and deaths. Importantly, 56% of cases and 63% of cancers and deaths were in the developing world. Of the estimated 371 000 new cases of cervical cancer in 1990, for example, around 77% were in developing countries. This latter case likely reflects socioeconomic pressures and the prevalence of causal factors such as certain strains of the human papilloma virus (HPV). Globally, the most common cancer affecting women is breast cancer, followed by cervical cancer. However, in North America the most common cancer in women after breast cancer is lung or colorectal. Around 226 870 women are predicted to develop breast cancer in the United States in 2012 and around 226 160 men and women will develop lung cancer, and around 143 460 colorectal cancer (www.cancer.gov/cancertopics/commoncancers). The data for this period should soon be available. Predictions for global cancer make sobering reading: it is predicted that by 2030 there will over 21 million new cases and above 13 million deaths each year from cancer.

Race and gender also influence rates of cancer and this is graphically illustrated by data from 1999 from the United States Department of Health and Human Services. Some of the findings, such as lower rates of melanomas in men and women of Afro-Caribbean origin, attributed to inherent protection from UV exposure, are predictable. Others, however, are less so. Thus, although prostate cancer is the most frequent cancer in males, rates are 1.5 times higher in Afro-Caribbean men than in white men. Similarly, the leading cancer in women, regardless of race, is breast cancer, followed by lung/bronchus and colon/rectal in white women and colon/rectal and lung/bronchus in Afro-Caribbean women. Breast cancer rates are about 20% higher in white women. Multiple myeloma and cancer of the stomach are among the top 15 cancers for Afro-Caribbean women but not white. Recent data have become available for the United States from 2005, which shows that the rate of all cancers combined for black, white, Hispanic, Asian/Pacific islander, and Native American Indian are 591, 526, 406, 314, and 280 thousand per annum respectively (http://apps.nccd.cdc.gov/uscs/).

Towards a definition of cancer

> A definition of poetry can only determine what poetry should be and not what poetry actually was and is; otherwise the most concise formula would be: Poetry is that which at some time and some place was thus named.
>
> *Karl Wilhelm Friedrich Schlegel*

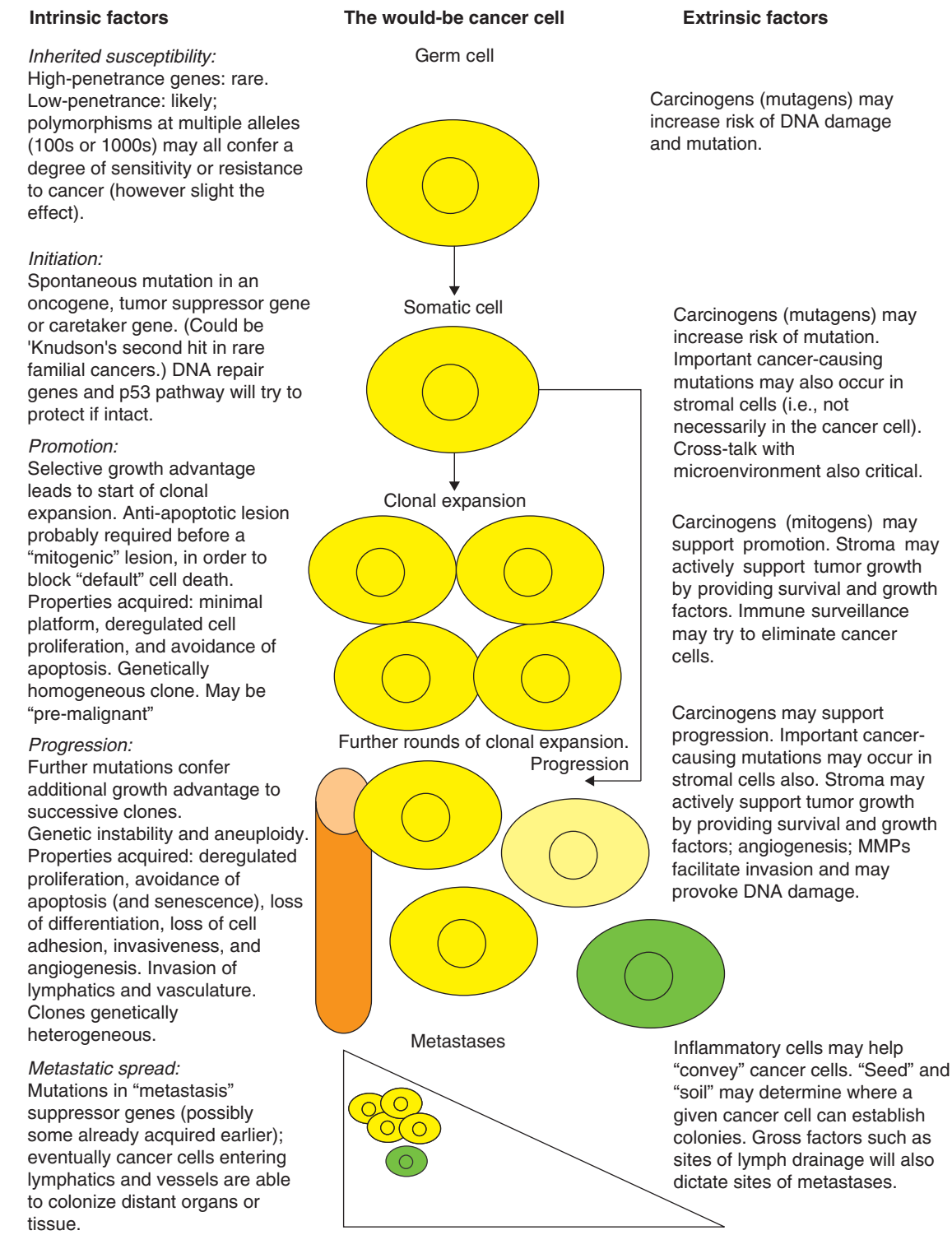


Figure 1.4 A highly stylized potential “life history” of a cancer cell. Cancer cells are shown in yellow (different shades denote subclones); stromal cells are green, vessels are orange.

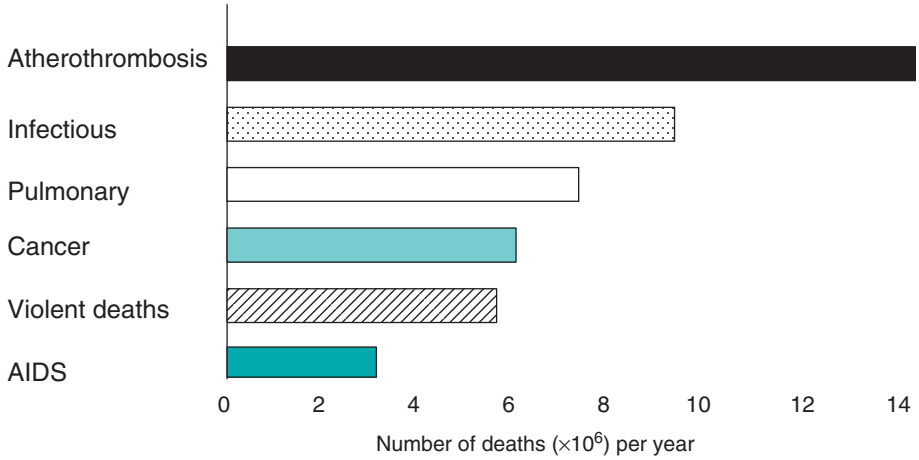
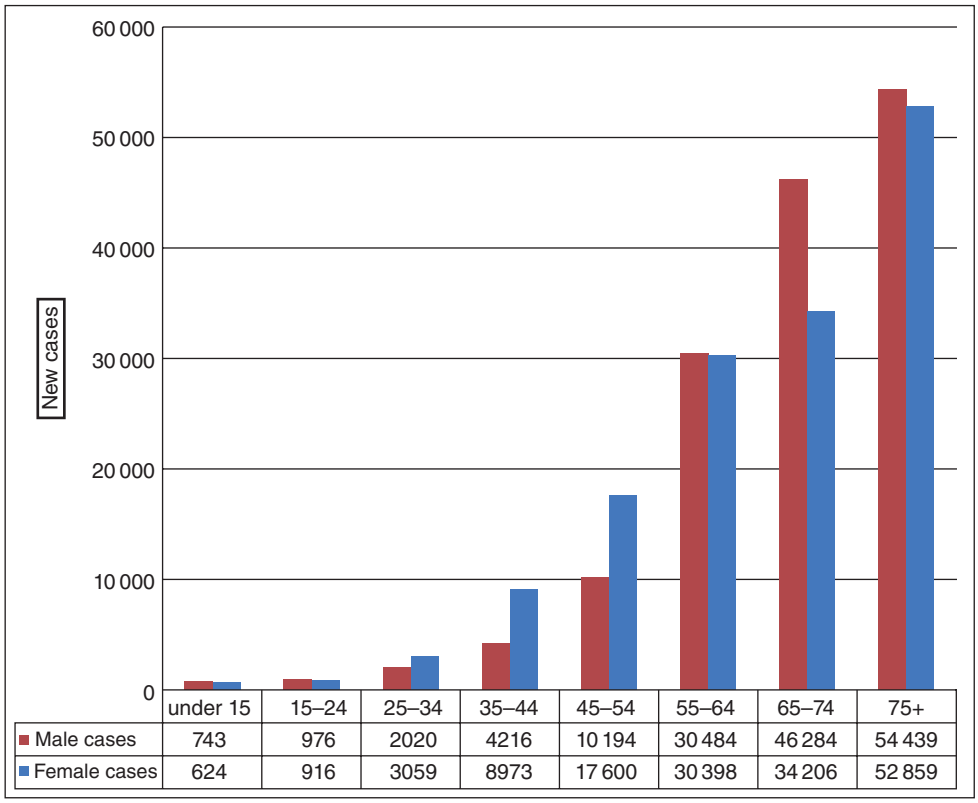


Figure 1.5 Leading causes of death worldwide, 2004. Redrawn from Murray CJL, Lopez AD (1997) Mortality by cause for eight regions of the world: Global. Burden of Disease Study. *Lancet*, **349**: 1269–1276.



	under 15	15–24	25–34	35–44	45–54	55–64	65–74	75+
Male cases	743	976	2020	4216	10 194	30 484	46 284	54 439
Female cases	624	916	3059	8973	17 600	30 398	34 206	52 859

Figure 1.6 Cancer development by age for 2007 in the United Kingdom. Modified from International Agency for Research on Cancer (IARC) and Cancer Research UK data.

The terms "tumor" or "neoplasm" are used interchangeably to describe a diverse group of conditions associated with uncontrolled cell replication. Tissue mass is normally tightly controlled to serve the needs of the organism. This control is achieved by the balancing of various and often opposing cellular processes (Fig. 1.7). Disturbing the balance of these processes results in diseases; if cell losses exceed renewal this results in degeneration/involution, whereas the converse results in tissue expansion, hyperplasia, or neoplasia. If the expansion in cell numbers is confined locally then it is described as "benign," but if this unscheduled cell replication is accompanied by invasion of surrounding tissues or spread to distant sites ("metastasis"), then it is unambiguously described as malignant.

These terms are relatively straightforward as they are descriptive and based on gross observations. It should be remembered, however, that the pathological definitions of benign and malig-

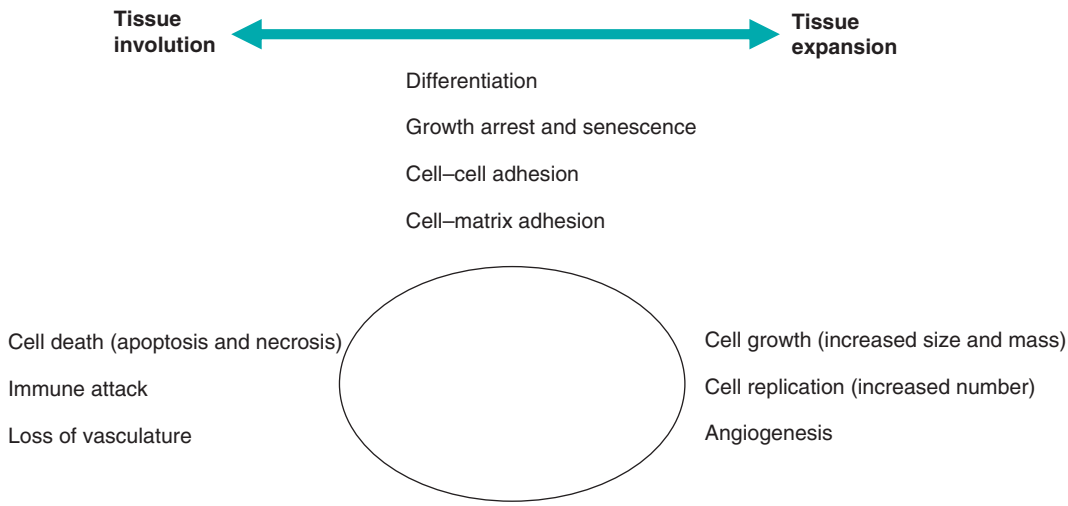


Figure 1.7 Processes contributing to regulation of tissue mass. Cell mass is determined by the balance of various cellular processes including, at the two extremes, growth/replication and cell death.

nant do not always translate into similarly benign or malign outcomes for the patient. Thus, a benign brain tumor causing severe neurological disturbance may be inoperable or require potentially life-threatening surgery, whereas a malignant prostatic cancer or microscopic metastases may have had no clinical impact and be discovered accidentally at post mortem. Adenomas are benign tumors originating in glandular or secretory tissues (such as lactotroph adenomas of the pituitary, which secrete prolactin, or parathyroid adenomas, which secrete parathyroid hormone – PTH). Such adenomas can result in substantial morbidity as a result of deregulated secretion of hormones and may also progress to become malignant, when they are termed "adenocarcinomas."

Classification of cancer

Classification of cancer is complicated by the variety of human cancers, with hundreds of different tumor types arising from almost every tissue and in every organ. This is further complicated by the ability of a cancer cell to invade surrounding tissues and metastasize to distant organs. Cancer biologists and oncologists have agreed on a classification based on the tissue of origin, regardless of organ location, focusing on the similarities in cellular structure and function among these tumors. Tumors are generally classified as either liquid or solid. The former includes leukemias and lymphomas comprising neoplastic cells whose precursors are usually motile. Solid tumors comprise either epithelial or mesenchymal cells that are usually immobile. Pathologically, cancers are classified as:

- **carcinoma**, originating from epithelial cells in skin or in tissues that line or cover internal organs and typically represent over 80% of human cancers;
- **sarcoma**, originating in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue;
- **leukemia**, a cancer originating in blood-forming tissues, such as the bone marrow, causing large numbers of abnormal blood cells to be produced and enter the bloodstream; and
- **lymphoma**, originating in the cells of the immune system.

It is worth emphasizing that the purposes behind disease classification are to help make the most accurate predictions about prognosis and response to particular therapies in the clinic and in the laboratory to ensure that as far as is possible like is studied alongside like. As discussed later, this objective may increasingly be better served by grouping cancers on the basis of their shared molecular pathoetiology rather than by tissue of origin.

"Carcinoma *in situ*" refers to lesions regarded as cancer that remain localized to the tissue of origin, often constrained by intact basement membrane. Such tumors often respond well to treatment, with good prognosis for the patient. In contrast, "invasive carcinomas," by disrupting basement membranes and growing into surrounding tissues, are more difficult to treat successfully. In addition, since invasion is usually a prerequisite for metastasis, the ultimate cause of most cancer-related deaths, even when the local lesion is treated, the prognosis is often poor.

Importantly, disease classification is not written in stone, as technical advances are made and larger numbers of individuals with a given disease are studied, it is often possible to recognize previously unappreciated "subclasses" of disease that can readily be detected and further improve accuracy of prognosis and prediction of treatment responses. Most recently, advances in postgenome era technologies such as oligonucleotide arrays and proteomics (Chapter 20) are allowing a subclassification of cancers in terms of molecular profile termed "tumor fingerprinting." At the same time genomics (essentially reading the DNA) is being increasingly applied to look for cancer susceptibility genes in patients and for mutations in tumors. It is hoped that in the future such powerful tools will ultimately allow more accurate determination of prognosis and even "tailored" therapy, whereby each patient can be uniquely classified and treated on the basis of such tests. These aspirations are often referred to as "individualized medicine", reflecting the ideal of being able to treat each individual in a uniquely appropriate way, based on variation in one or more of the following parameters: gene alleles, gene expression/protein expression and mutations in tumor cells, proteins in the blood.

It is surprisingly difficult to define cancer in practice

Cancer is a difficult term to define accurately. Put simply, cancer is synonymous with malignancy, and refers to a group of conditions that have manifested malignant behavior, namely

Box 1.1 Cancer screening

In 1968, Wilson and Jungner of the WHO set down ten principles that should govern a national screening program:

1. The condition is an important health problem.
2. Its natural history is well understood.
3. It is recognizable at an early stage.
4. Treatment is better at an early stage.
5. A suitable test exists.
6. An acceptable test exists.
7. Adequate facilities exist to cope with abnormalities detected.
8. Screening is done at repeated intervals when the onset is insidious.
9. The chance of harm is less than the chance of benefit.
10. The cost is balanced against benefit.

The aim of screening is to identify at-risk individuals for whom effective interventions or treatments are available, and should also be limited to situations where that treatment is more effective if administered early and before the condition to be treated becomes readily apparent. If the above criteria are satisfied, then in general, the ideal screening test for any given condition should be highly sensitive (few false negatives – patients deemed normal who actually have the condition) and highly specific (few false positives – normal patients deemed to have the condition). In many cases, increasing sensitivity may result in decreasing specificity, and often health policy decisions have to be made that take account of the prevalence and severity of the condition to be screened for, economic factors relating to the cost of screening and the subsequent proposed interventions, and also both efficacy and the safety of the available interventions (risk/benefit ratio).

Broadly, two types of screening are applied: (1) **population screening**, where mechanisms are put in place to ensure that all appropriate individuals are screened at given times/intervals – largely the responsibility of public health organizations; (2) **opportunistic screening**, where healthcare workers undertake screening when individuals present to them for whatever reasons – this is largely the responsibility of healthcare professionals. The latter approach is cheaper, but will provide less cover of the population.

In the vast majority of cancers there is little doubt as to the potential severity of the condition, and in some cases where the treatment offered is fairly innocuous (e.g. lasering of cervical lesions) one can afford to treat a number of so-called false positives. However, if the treatment involves bowel resection or mastectomy, for example, this calls for much greater accuracy in prediction and a smaller number of false positives can be accepted. In clinical practice this is often reflected in how early in the evolution of a potential cancer such treatments are offered and therefore also on the extent to which the given cancer can be prevented. In general, earlier is better, but this requires much greater ability to predict the behavior of a given tumor or lesion.

unscheduled and uncontrolled cell growth leading to invasion and/or metastases. There is no ambiguity in this case as the definition is "retrospective" and based on the readily observable behavior of the "cancer." Such a narrow definition is of limited practical value in the laboratory, however, and particularly in the clinic, as it precludes true preventative or even early treatment. This seemingly abstract issue is placed in context when it is remembered that for those cancers where rates of death have actually been reduced over the last few decades, this has resulted primarily from improvements leading to earlier diagnosis and earlier administration of treatment.

It is clear that certain features at a microscopic level can accurately be employed to identify a tumor as cancer before it manifests overtly malignant behavior clinically (metastasizes to lymph nodes or other organs or has on imaging or surgery been shown to have invaded local structures). In other words, a cancer is a cancer before it necessarily declares itself by behaving as one. In most cases, this requires the demonstration of evidence of penetration of a basement membrane or invasion into surrounding tissue (which means you need to look at a piece of tissue that includes the tumor – histological examination) and/or the presence of "cancer cells," namely cells exhibiting defined changes, which from experience are the same or similar to those seen in circumstances which are incontrovertibly cancer (which means you need to have acquired some cancer cells from body fluids, sputum, or via a smear- cytological examination). Clearly, the latter is often quicker and less invasive in clinical practice.

In a clinical setting, where the primary purpose is to identify a tumor or lesion that requires surgical excision or other treatment, it may be sufficient to know that a particular lesion (based on gross appearance or histological examination) poses a risk of proceeding to an invasive cancer. A lesion may already be regarded as a cancer, on the basis of abnormal growth or appearance and the near inevitably of progression to invasion (carcinoma *in situ*), or its potential may not be yet realized/manifested but risk of progression is high ("precancerous" or "premalignant"). This forms the basis for identifying "high-risk" lesions such as breast carcinoma *in situ,* Barrett's esophagus (a precursor of esophageal cancer), colonic polyps (a precursor of colon cancer), and others. Cytological examination may identify premalignant cells and is employed where such cells can readily be obtained, including cervical screening for the early detection and prevention of cervical cancer (Box 1.1).

For a research scientist, these distinctions are also of critical importance. The ability to define the point at which a premalignant benign lesion ends and a malignant cancer begins is a prerequisite to understanding the initiation and key early events in cancer formation. In the laboratory the progressive behavior of transformed cells or tumor progression can be investigated in animal models, as long as the necessary investigative tools are available, but this opportunity is self-evidently usually lacking in the study of cancer in humans. The cancer researcher can validate predictions made about the future behavior of a given lesion by prospectively tracking the eventual emergence of invasive metastatic cancer but, as will become clear later, the actual stage of evolution at which cancer cells emerge and acquire ability to become invasive and metastasize is still contentious and quite difficult to detect.

Cancers may not always be clinically apparent

Difficulties of definition notwithstanding, the clinical situation is further complicated by the increasing awareness that microscopic colonies of cancer cells (*in situ* tumors) can be detected in different tissues (thyroid, breast, prostate for example) at autopsy in most older individuals. In fact, such clinically irrelevant *in situ* cancers may be a 100- to 1000-fold more common than clinically apparent cancers arising in those same tissues during life. For example, most older individuals have *in situ* thyroid carcinomas at autopsy, whereas only around 0.1% of similarly aged individuals are found to have thyroid cancer during life. Although biologically intriguing and testifying to the potential effectiveness of innate anticancer defenses (such as antiangiogenic factors), such findings may increasingly be problematic in the clinic.

Until recently, we have generally not detected the vast majority of such *in situ* tumors during life, largely because we do not routinely biopsy tissues in apparently healthy individuals. However, one area in which detection of such *in situ* tumors may pose difficult and as yet unresolved clinical dilemmas, is increasing use of diagnostic prostatic biopsy in older men, and discovery of so-called "incidentalomas" during routine imaging procedures such as CT and MRI scanning. The now ubiquitous presence of privately run "walk-in" imaging centers offering the dubious benefits of whole-body scans will undoubtedly compound this problem. For example, what do you do about the incidental lump that is not self-evidently cancerous – particularly as benign irrelevant lesions will be considerably more numerous? The patient will likely be anxious and may well push to undergo potentially dangerous invasive diagnostic steps in order to be as certain as possible that they do not have cancer. Guidelines have had to be developed and will continue to be needed to assist clinicians in deciding which individuals with such findings actually require any form of treatment or just reassurance.

However, this is far from straightforward, as there are many cases where the actual ability to predict the risk of future invasive cancer based on the appearances of a given lesion are not yet sufficiently mature. A good example is the readily visible dysplastic white lesion in the mouth that may in some cases – but by no means all – herald the development of an oral squamous cell carcinoma. Ironically, at least in some cases, where the lesion may be less likely to come before the eagle eyes of dentists and GPs or it is not technically possible to detect the early lesion let alone examine it, this may be for the best until our ability to more accurately predict the future behavior of these early lesions improves and/or we greatly increase our current arsenal of sufficiently well-tolerated and nonharmful therapies to exploit the potential benefits of early diagnosis.

This interesting debating point notwithstanding, it is abundantly clear that in order to prevent or cure cancer effectively it is essential to diagnose disease as early as possible, and nothing should distract us from our efforts to progress in this goal. Failure to do so will inevitably mean that potentially life-saving early treatment for some individuals destined to develop clinically important cancer will be delayed. To resolve this conundrum is theoretically simple – we just need to distinguish early lesions that will never progress to disease from those that will progress to cancer. Although, routinely screening apparently healthy individuals for certain cancers has been well-validated and has become accepted best practice for cancers of breast (mammography), cervix (Pap smear), and colorectum (fecal occult blood) in many countries, for most cancers we urgently need better tests and tools. Fortunately, the research community has responded to this challenge and much progress is being made in finding new tests and "biomarkers" for various cancers that might give important information about prognosis and treatment response (see below and Box 1.2).

Box 1.2 Cancer biomarkers

Leland Hartwell, in his keynote address at the 2004 meeting of the American Association for Cancer Research (AACR), suggested that earlier diagnosis and improved monitoring of cancer progression by noninvasive means could dramatically improve the outcome for many patients. Early detection represents one of the most promising approaches to reducing the growing cancer burden and has been revolutionized with the advent of postgenome era technologies that can identify cellular changes at the level of the genome or proteome and new developments in data analyses and modeling. Gene expression profiling of various human tumor tissues has led to the identification of expression patterns related to disease outcome and drug resistance, as well as to the discovery of new therapeutic targets and insights into disease pathogenesis. However, techniques requiring removal of cancer tissues can only be employed once a tumor has been detected and are unsuitable for earlier diagnosis and for general screening. A noninvasive test would have numerous advantages. Therefore considerable efforts are now directed at finding biomarkers in blood tests. These are obtained relatively noninvasively and rapidly, and could be employed in screening. Biomarkers could also be useful in posttreatment follow-up for disease recurrence. Most current tumor biomarkers are lacking in sensitivity and specificity, and more effective ones are required.

Therefore, considerable efforts are now directed at finding "biomarkers" in blood or urine tests that can be obtained relatively noninvasively and rapidly, and could much more readily be employed in screening large numbers of individuals. Their role could also be extended into surgical surveillance for potentially operable disease and postoperative follow-up for disease recurrence.

Broadly, three overlapping technologies can be employed to look for cancer biomarkers:

1. Analyses of proteins by: (a) immunoassay of single known proteins predicted to be of interest; (b) proteomics, including 2D gel-based separation or liquid chromatography followed by mass spectrometry to identify potentially thousands of different proteins; (c) proteomic pattern analysis or "fingerprinting," which relies on the pattern of proteins observed and does not rely on the identification of individual traceable biomarkers.
2. Analyses of free RNA, including miRNA, in the circulation some of which derives from the cancer.
3. Isolation and study of circulating tumor cells, which can in turn be profiled for gene expression by microarrays.

As mentioned earlier, in order to improve our predictive/diagnostic abilities, traditional examination of patients in the clinic, application of imaging techniques, and cytology/histology of the tumor are increasingly being supported by newer techniques, such as molecular profiling. Traditionally, genetic analysis looks for single susceptibility genes that confer a high risk of cancer formation, but in future this may include more complex genomic testing (of multiple polymorphic alleles – see below), or direct analyses of gene/protein expression in the tumor by various techniques including gene chip microarrays and proteomics. Considerable enthusiasm has been generated by the possibility of using relatively noninvasive tests to identify cancer biomarkers in blood samples or other body fluids from patients with cancer or at risk of cancer. Thus, proteins, mRNA, or miRNA derived from the tumor or from the body's response to it might be analysed in body fluids. In many cases it has also proved straightforward to isolate and examine cancer cells (or their DNA) from blood or topically. If such information can be correlated with the presence or absence of cancer in the healthy population, or with clinical outcome or treatment response in known cancer patients, then these will be useful biomarkers.

The best-known currently used serum biomarker is prostate-specific antigen (PSA), elevated levels (or progressively rising levels) of which are associated with significant risk of prostate cancer. However, this falls short of the ideal in several respects, in particular the number of false positives (the test wrongly suggests the possibility of prostate cancer) and false negatives (a cancer fails to be diagnosed). This means that even clinical trials disagree on the benefits of general screening with PSA. In fact, there is a more fundamental flaw in the notion of simply detecting presence of prostate cancer by screening: it gives no insights into prognosis. This quandary is easiest to appreciate if we assume an ideal performance for the test and thus have in some way eliminated false positives (without compromising sensitivity). So now we use the test and it unambiguously tells us which patient has prostate cancer. What it does not tell us is what to do with the patient. Why not? Because recent trials have suggested that PSA screening results in overtreatment because the prognosis of occult prostate cancer is so variable and often does not affect mortality or morbidity (see Chapter 3). This does not mean that PSA screening is without value, it makes a major contribution to the investigation of patients with symptoms of prostatic enlargement (difficulty in micturition) and in the follow-up of prostate cancer patients following treatment. However, how suitable PSA is for screening the general population is controversial.

Tumor-derived biomarkers are already in routine use in the clinic. Thus, the presence of estrogen and progesterone receptors or of a *HER2* mutation in breast cancer defines patients who will likely benefit from hormone-based therapies or trastuzumab, respectively. Commercial biomarker assays which measure expression of multiple genes and mutant versions from tumor samples by reverse transcription polymerase chain reaction (RT-PCR), such as the Oncotype DX test for breast cancer (measures *HER2*, *ER*, and *PR* status as well as 13 other cancer-relevant genes including Ki-67 and survivin) are now available. The presence of mutant *KRAS* in a colorectal tumor identifies a subgroup who will respond poorly to drugs targeting epidermal growth factor receptor (EGFR).

Genotype may also be helpful. Thus, a recent large study has confirmed that breast reduction surgery in women who are carrying germline mutations in *BRCA1* and *BRCA2* can markedly prevent breast cancer in these individuals. In a recent study, there were no diagnosed cancers in 247 women with risk-reducing mastectomy compared with 98 women of 1372 diagnosed with breast cancer who did not have risk-reducing mastectomy. Moreover, women undergoing risk-reducing salpingo-oophorectomy had improved survival.

If we find a cancer what do we do with it?

Not only do we often not know who to treat, we are often unsure what treatments to use, particularly before the development of an obviously life-threatening cancer. This situation has not been helped by the fact that the majority of therapeutic trials have focused largely on the end stages of cancer, by definition, the point at which these therapies are least likely to successfully cure the disease. Why? Because regulatory approval requires a lengthy series of clinical trials (see Chapters 15 and 16) and these are most readily conducted in patients with advanced cancer for whom no further treatments are available. Use earlier in the disease process is often left until after marketing and then interpretation may be confused by the need to use the new drug alongside existing best practice.

Improved ability to predict treatment response is fundamental to avoiding the morbidity and mortality associated with cancer while also restricting potentially harmful or even life-threatening treatments to those individuals most likely to benefit. Most treatments are justifiable when a life-shortening cancer is prevented, but would be very undesirable if employed in an individual never destined to develop cancer and who will eventually die of some unrelated other cause and whose life would have been affected less by the cancer than by the treatment. In practice, what is needed are clinical measures or new biomarkers that correlate with prognosis and that ideally also assist in selecting the best treatment or combination of treatments (from among watchful waiting, surgery, radiation, and drugs).

One thing is clear: early treatment offers the best chance of a successful outcome. This problem is addressed by various screening programs aimed at identifying premalignant or early stage cancers (see Box 1.1). Importantly, in these cases suitable treatment strategies have been defined.

As discussed in the previous section, it is hoped that detailed molecular analyses of tumor samples or body fluids will not only improve our understanding of the "roadmap" to cancer for any given cancer, which might in turn guide us to the application of specific drugs to target particular genes/proteins, but may also improve our ability to predict therapeutic responses. Such detailed analysis of individual tumors starts to realize the potential of post-genome era science and may finally deliver the ultimate goals of "individualized medicine" and "tailored therapy" – where treatment is fitted specifically to an individual.

The best treatment is prevention

Prevention requires a combination of activities involving different organizations, including public health strategies aimed at the whole population and exemplified by activities targeting adverse lifestyles, including smoking and poor diet. More targeted advice and possibly interventions may be needed for individuals at the highest predicted risk of disease. A new discipline of chemoprevention has been established with the sole purpose of designing the perfect weapon for a pre-emptive strike against future cancer cells. However, with the rare exceptions of individuals with known familial cancer syndromes, this has proved far more dif-

ficult a strategy for cancer prevention than it was for preventing coronary heart disease (CHD); there are no statin equivalents for cancer prevention.

At one extreme, no complex tests are needed to spot obese patients and smokers, and accurately predicting which of these will get early cancers as a result may be unnecessary, because encouraging all to change behavior appears a reasonable approach, particularly as in these cases such a lifestyle treatment is not likely to have any "off target toxicity" (prevent cancer but cause something as bad or worse). In other words, assuming that everybody is at risk of smoking and obesity-related disease may be good enough. Recent guidelines have placed vaccination of young women to prevent cancer-causing infection with HPV in this category. Being female is considered a sufficient risk of being infected with HPV and developing cervical cancer in the future and no subclassification is deemed desirable. Indeed, further selection could actually compromise the efficacy of vaccination as it might reduce the chance of developing herd immunity, although one could use sexual activity as an additional screening test and restrict vaccination to the noncelibate. In the case of invasive treatments, clearly it is preferable to narrow down the risk much more before offering preventative surgery or toxic drugs unless these are targeted correctly. Thus, before offering mastectomy to a woman to prevent breast cancer we need to know a lot more than just her gender. In this case the presence of very high-risk mutations in *BRCA1* and *BRCA2* identify a small subset of women who will benefit from surgery. However, there really is not much in between these two extremes. In other words, for most of us there are no simple tests that can be used to predict our risk of future cancer.

Robust tools have been developed allowing reasonably accurate estimation of future risk of heart attacks or strokes based on using simple information such as age, sex, blood pressure, and level of circulating fats (readily determined in the clinic) in order to calculate a risk score. For cancer the hope is that improved genetic testing (Box 1.3), measurement of new disease biomarkers, and improved clinical investigational tools will match these successes in time. Screening is discussed in more detail below and in Box 1.1.

What is next best?

The early detection of cancer or precancer syndromes is self-evidently the next best to prevention, based on the assumption that small numbers of well-localized cells of a potentially less advanced state of malignancy will prove easier to treat or cure. This forms the basis of screening for cervical, breast, and colon cancers (Box 1.1). Improved early detection also involves the speedy selection of patients with appropriate symptoms or signs for early application of diagnostic tests (including X-rays, blood tests, biopsy, etc.). The nature of such tests is continually evolving, with great hope placed on the identification of cancer biomarkers and resultant possibility of molecular diagnostics gradually supplanting or complementing more traditional morphological assessments. Biomarkers may derive from a variety of sources, including serum proteins or nucleic acids, circulating cancer cells singly, or as part of complex molecular signatures. Not all such new diagnostic tests will necessarily result from ever more advanced molecular and cellular biology. Some of the ideas of the original pioneers of cancer biology still have potential and are being evaluated (see Jean Astruc, in Appendix 1.1), even highly creative or eccentric ideas such as training "sniffer dogs" to identify bladder cancer from the smell of a person's urine (though with any dog I've met the trick appears to be to stop them publicly "screening" everybody within reach!).

Box 1.3 Genetic testing

The identification of disease-related genes has led to an increase in the number of available genetic tests that detect disease or an individual's risk of disease. Gene tests are available for many disorders, including Tay–Sachs disease and cystic fibrosis, in cancer testing for the *BRCA1* genes and breast cancer, *MEN1* and *RET* in endocrine tumors, and as more disease genes are discovered, more gene tests can be expected.

Currently available treatment options

The number of treatment options has expanded dramatically in recent years with the emergence of specific therapies targeting individual cancer-relevant molecules or signaling pathways. Thus, knowledge that a cancer is possessed by a particular malign oncogenic mutation can be exploited by the administration of a suitable therapeutic exorcism. However, choice of appropriate treatment regimens for any given patient remains challenging. In general, the first decision to be made is whether the cancer may be cured by surgical resection and radiation or drugs, or both. A more detailed discussion of cancer therapies is presented in Chapter 16, but a few interesting aspects will be highlighted here.

Achieving lasting remission in patients suffering from nonlocalized malignancies remains elusive. We are rarely, if ever, able to kill all the cancer cells in the primary tumor and metastatic lesions. Such failures may be the result of poor access of effective treatments to all tumor locations, varying susceptibility to conventional DNA-damaging anticancer agents, or the rapid evolution of resistance. A particular problem is posed by cancers where cells spread early via the circulation to establish micrometastases in the bone marrow or elsewhere. While increasing drug dosage can overcome some of these barriers it also increases toxicity to normal cells; to paraphrase Paracelsus, "The dose makes the poison."

Traditional cytotoxic treatments aim to kill all cancer cells, whereas some newer approaches may be directed at disabling cancer cells (inducing growth arrest, differentiation, etc.), without necessarily killing them. Therapeutic resistance is a major issue in cancer treatments and may arise by cancer cells acquiring new routes of signaling that bypass the drug-targeted protein or even by developing ways of blocking the drugs access to the cell. Cancer stem cells are also a potential explanation for treatment resistance and recurrence, as such cells may be inherently more resistant to agents or may occupy environments such as hypoxic niches which protect them.

Despite some notable successes, concerns remain about potential adverse effects of traditional radio- and chemotherapy on normal tissues, and intriguingly also on the surviving cancer cells themselves. Cancer progression is an evolutionary process driven by acquisition of epimutations, which provide a selective growth advantage to particular cell populations. Therapies that induce irreparable damage to cell DNA may have undesirable consequences on cancer cells – they may fail to undergo apoptosis and

go on to survive the onslaught. In fact, one mechanism of resistance in cancer cells may be the increased mutation rate and selection pressure provided by such drugs. The net effect of unsuccessful cancer therapies could be to speed the progress of the disease, as more mutated cells expand without the competition of their less aggressive predecessors and their offspring. We might actually help to select for more aggressive clones or enrichment of more malign cells such as CSCs. Increasingly, therefore, new combinations of drugs are employed to reduce the likelihood of cancer cells surviving to become resistant to all these agents.

An interesting parallel may be drawn here between evolution of species and evolution of a cancer. Evolution is driven not only by mutations and natural selection, but also by catastrophic extinctions which, by removing less-hardy competitors, clear the path for the survivors to fill the vacuum. It may be that subtotal cancer cell killing with chemotherapy, radiotherapy, or even surgery is the cancer equivalent of a meteor impact. Given, that only some 1 in 10 000 of the estimated 50 billion or more species that have evolved on earth still exist, and that if we are anything to go by the survivors include some of the hardiest and nastiest, then perhaps extinctions of some of the less able to survive may be undesirable if you do not cull the lot. Moreover, the situation in cancer therapy is likely a lot worse, as cancer cells are repeatedly selected for their ability to not be killed by cancer therapy, whereas species have not necessarily been selected largely on their ability to survive repeated meteor impacts or volcanic activity but probably somewhat more randomly. The risks inherent to increasing "selection pressure" have been ably demonstrated in the case of emergence of antibiotic resistance in bacteria.

So what might be an effective alternative to treatments involving chemo-, radiotherapy or surgery? Theoretically, arresting replication in cancer cells might be a good alternative or addition to traditional treatments that offer anything other than complete extinction of cancer cells, as this would prevent expansion of an aggressive surviving clone and might instead foster "stagnation" of the cancer cell population. Assuming that no treatment will ever immediately kill all cancer cells – what proportion effectively constitutes total extinction? The 90% extinction of species believed to have occurred in the Permian era was followed by a substantially slower recovery (based on fossil records and therefore really only applies accurately to "big organisms") than after those in different eras which resulted in 60–70% extinction – but they still eventually recovered. Arguably, we might wish to know what proportion of cancer cells need to be killed in an individual for no symptomatic recurrence of the tumor to take place during that individual's lifespan!

Causes of cancer

Much has been learned about the causes of cancer, including the role of genetic predisposition, gene–environment interactions, and infectious agents. Intriguingly, recent research points to the considerable overlap between the behavior of cancer cells and that of cells during normal physiological wound healing and during embryogenesis. Similarities include replication, less differentiated state, invasion/migration, with the major differences reflecting the lack of control and the unscheduled nature of replication that characterize cancer. One intriguing question, addressed later, is how the organism is able to distinguish between normal growth and tissue repair (normal cell cycles) on the one hand and neoplastic growth (cancer cell cycles) on the other.

The clonal evolution theory

Most cancers derive from an individual somatic cell in the adult organism, with the initiation and progression of tumorigenesis dependent on the accumulation of genetic or epigenetic changes that determine the emerging cancer phenotype. Initiation is believed to be through DNA damage, which renders the cell capable of forming a cancer; initiated precancer cells then multiply during a promotion phase. One way of looking at this is to assume that the first mutation in some way liberalizes the would-be cancer cell, thereby generating an underclass of uniquely susceptible cells among which some may subsequently become increasingly radicalized. Cancer cells are created by the assimilation of epimutations that promulgate increasingly individualistic and sociopathic behaviors at odds with the best interests of the organism and, moreover, this process may proceed through recognizable stages.

A "multistage" model of carcinogenesis, based largely on epidemiological observations, was first articulated by Peter Armitage and Richard Doll in the 1950s. The rapid expansion of knowledge about the molecular genetic basis of disease then allowed Nowell, in 1976, to suggest that cancers arise by a process of multistep clonal evolution. He proposed that most neoplasms arise from a single cell of origin, and tumor progression results from acquired genetic variability within the original clone, allowing sequential selection of more aggressive sublines. He also stated, rather prophetically, that acquired genetic instability may result in apparently similar individual advanced tumors being very heterogeneous both at a molecular and behavioral level and might require individual specific therapy. He also predicted that therapy could be thwarted by the emergence of genetically variant resistant sublines.

Becoming a cancer cell – multistage carcinogenesis

A wealth of data has supported the view that cancers are multistage diseases progressing via protracted accumulation of multiple genetic and/or epigenetic changes (lesions) that compromise control of cell proliferation, survival, differentiation, migration, and social interactions with neighboring cells and stroma.

Hanahan and Weinberg have recently updated their seminal review of 2000, in which they originally construed the axiomatic requirements of cancer cells as:

(1) the capacity for self-sufficient proliferation, independent of exogenous growth signals;
(2) refractoriness to growth inhibitory signals;
(3) resistance to apoptosis;
(4) unrestricted proliferative potential (immortality);
(5) capacity to recruit a vasculature (angiogenesis);
(6) ability to invade surrounding tissue and eventually metastasize.

Not surprisingly, other cancer-critical processes have been vying for the dubious accolade of becoming the seventh hallmark feature of cancer ever since. The Warburg effect, a shift in energy production from oxidative phosphorylation to glycolysis, is currently leading the polls, but is being hard pressed by avoidance of immune surveillance, tissue remodeling, and a variety of forms of stress or stress phenotype. In most cancers the presence of chronic inflammation alongside subversion of expected interactions with immune and stromal cells also appear to be common features.

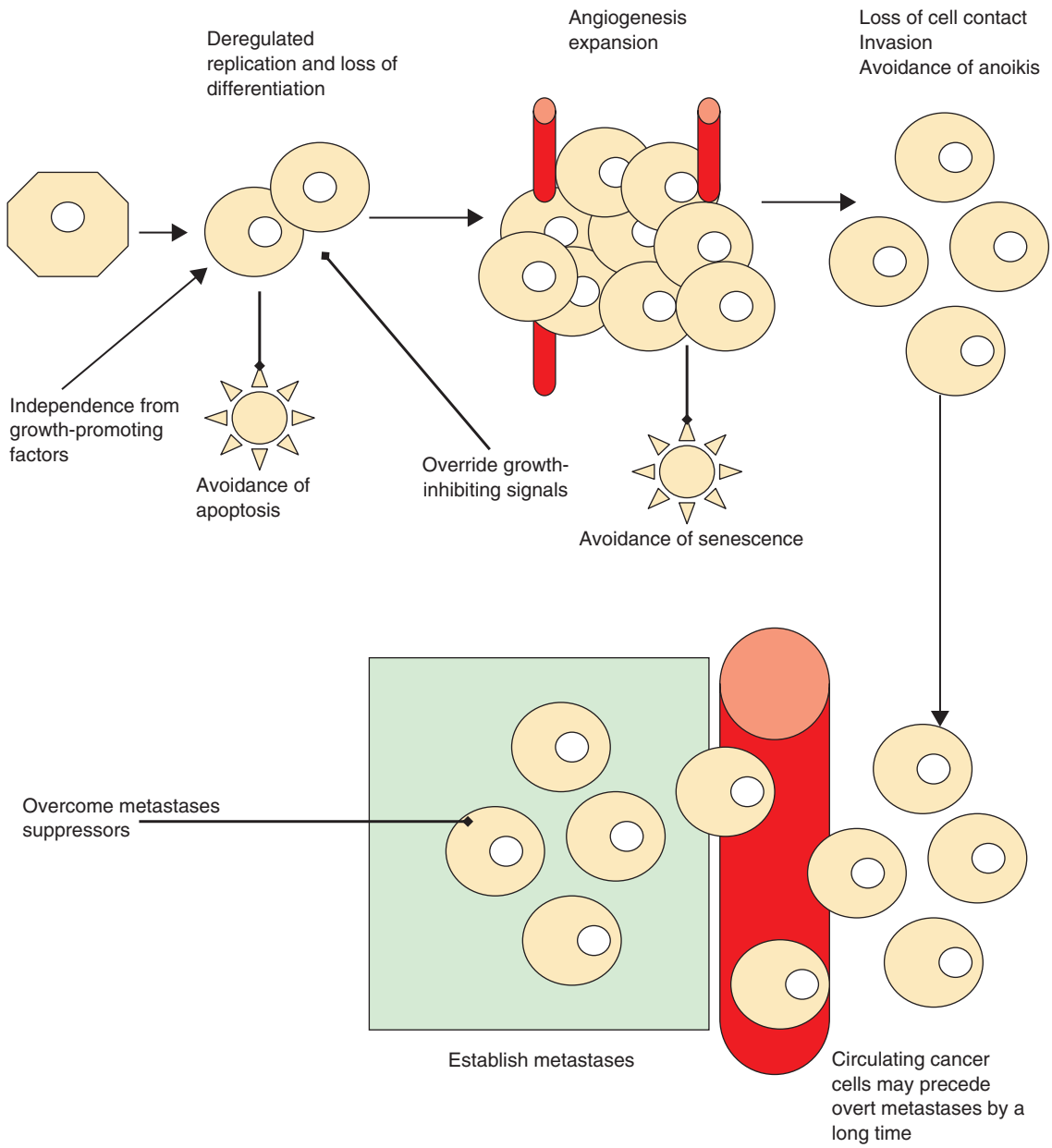


Figure 1.8 Processes contributing to cancer formation. The "hallmark" features of cancer are shown, appearing in a potential sequence. It should be noted that this does not imply that this is the actual sequence in which such features are acquired in any particular cancer.

By implication, tumor progression proceeds by the acquisition of lesions that provide the tumor cell with these attributes and which thereby shape the complex phenotype of the tumor cell (Fig. 1.8). Mostly, these lesions are acquired in somatic cells, but in the inherited cancer syndromes (see Chapter 3), one of the lesions is inherited and is present in all somatic cells – the would-be cancer cell has a headstart in life. It is important to note that seemingly phenotypically similar cancers may arise through differing combinations of lesions: there are likely many different routes to cancer, even in the same cell type (Box 1.4). Many key cancer-relevant signaling pathways may be activated or inactivated by mutations at various different points that could result in largely identical cell behaviors. Many of the "hallmark" features of cancer cells may be the consequence of reactivating embryonic developmental programs by different routes and will be discussed later with respect to CSCs and EMT.

Genetic alterations conferring the hallmark features generally involve gain-of-function mutations, amplification, or overexpression of cancer-driving genes (oncogenes) or loss-of-function mutations, deletion, or epigenetic silencing of cancer-restraining genes (tumor suppressors) or DNA-repair genes (caretakers). Although the genes involved show considerable overlap between individual patients and types of cancers (mutations in some 14 or more genes, including *RAS*, *RB*, *p53*, *PI3K*, are frequent offenders) they are found alongside a wide variety of other much more "individualized" low-frequency alterations involving several hundred distinct genes that give each tumor its often unique blueprint.

Box 1.4 Two steps to seven? The roadmap for cancer

"Pluralitas non est ponenda sine necessitate."

William of Ockham, the most influential philosopher and theologian of the fourteenth century, is best known for applying the medieval rule of parsimony to formulate one of the best-known principles of science, Ockham's razor: *Pluralitas non est ponenda sine necessitate*, translated as "entities should not be multiplied beyond necessity." As a principle in science this may be expressed as "favour the simplest model which explains the observations." Even earlier, Aristotle pointed out that "nature operates in the shortest way possible."

It has been widely assumed that since (i) human solid tumors when examined carry a plethora of genetic and epigenetic alterations and (ii) it is genetically difficult to transform cells under tissue culture conditions, cancer formation can only occur under the influence of multiple (possibly 7 or more) genetic lesions. However, in some cases the situation may be much simpler. Namely, that the key requirements for tumorigenesis are deregulated cell proliferation and suppression of cell death, and that mutations enabling these may constitute the "minimal platform" for the development of a cancer, at least where one of those lesions is deregulated expression of c-*MYC*. It is clear that there are far fewer "pathways" implicated in cancer than genes. Therefore, some cancer cells may indeed "arrive" at this destination via a protracted route involving multiple mutations, as the way in which a given cell activates or suppresses the requisite pathways needed to complete this "journey" may be very variable.

Some of the pathways strongly implicated in cancers include those regulating G_1/S transition in the cell cycle, including the Rb protein, the p53 tumor suppressor pathway and other apoptosis pathways, and the angiogenesis/HIF1 pathway. In fact, there are now numerous examples of only two genetic lesions fulfilling these requirements and promoting neoplastic progression, suggesting that at least in some cases the genetic basis of a given cancer may be remarkably simple. In this model, the genetic complexity of an advanced tumor is more a reflection of evolutionary pressures and natural selection of clones with a growth advantage, rather than an indication of the mutations required to initiate that tumor. The "mission critical" mutations are concealed within the plethora of mutations, many of which are likely irrelevant to tumorigenesis.

This minimal platform model may be reconciled with studies of cell transformation *in vitro* – it may be much harder to establish transformation and immortality in a cultured cell than to produce a cancer cell within the organism. The intact organism comprises a network of usually highly effective anticancer barriers, but once these become breached they may instead support the developing tumor. This is not pure conjecture, it is clear that the organism provides the developing tumor with a blood supply as long as it is instructed to do so; in some cases this may require an "angiogenic switch" (an acquired mutation which allows the tumor to "request" to stromal cells for angiogenesis), but might also be an inevitable accompaniment of tissue growth, no matter how inappropriate. In fact, much is now known about the interactions between proangiogenic factors produced by the tumor (such as FGF, VEGF, and PDGF) and antiangiogenic factors produced in the tissues or within the circulation (such as thrombospondin, tumstatin, endostatin, angiostatin, and interferons alpha and beta, respectively). The initiation of angiogenesis is likely dictated by the balance of these factors, and in turn by the genes expressed by a given cancer cell on the one hand and by the tumor microenvironment on the other.

The multistage theory of cancer formation is illustrated by models proposed by Eric Fearon and Bert Vogelstein to explain the observed behaviors of carcinogenesis in the colon (see Fig. 3.3). A normal colonic enterocyte acquires a mutation that confers a growth advantage and begins to expand clonally. This stage may be protracted as the progression to full malignancy may require not just one mutation, but between 8 and 12 independent mutations. The chances of a single mutation occurring among the billions of gut cells over a 70-year or more lifespan is substantial. However, the chance of two mutations occurring in one cell is much less (the square of the original probability) and for all 8 or more mutations to occur in one cell in the lifetime of an individual is vanishingly small. However, if one also assumes that each mutation results in clonal expansion, then these odds begin to narrow rapidly (a second mutation is clearly going to be more likely in a few million proliferating cells than in one).

An alternative explanation for the infrequency of cancer development is that interlocking combinations of mutations might be required from the outset; in other words, more than one mutation is needed for the initial expansion of a clone of cells. Recent work by several laboratories has supported this notion by finding that in certain cases the mutational route to cancer is rather short (in molecular terms), with as few as two interlocking mutations required for initiation and progression of cancers in animal models, and – at least where one of these lesions involves particularly "dangerous" oncogenes such as c-*MYC* – also in humans (Box 1.4).

The cell of origin in cancer

In the 1950s, the histologist Charles Leblond described three main mechanisms by which adult organs are maintained: **static**, where essentially no replication occurs (e.g. nervous system); **self-renewal**, where stem cells compensate for rapid losses of differentiated cells (e.g. gut and skin epithelia, blood); and **simple duplication**, where tissues are maintained by proliferation of their own differentiated cells (tissues with slower turnover, such as pancreas, liver, kidney, blood vessels). Interestingly, this early view has been largely discarded in recent decades in favor of the notion that essentially all adult tissues are maintained primarily from a local minority subpopulation of progenitor cells, which retain a strong proliferative capacity, as well as the ability to differentiate into the required mature cell types after dividing – the so-called stem cells (see Box 5.2 – Stem cells). Only recently, with seminal studies employing direct lineage tracking using "pulse-chase" techniques (see Chapter 20), have experimental data actually provided unambiguous support for Leblond's original idea at least with respect to simple duplication being important in pancreas.

It is a widely held view that cancers originate primarily in stem cells. In fact, the stem cell origin of cancer originates from mid-nineteenth century microscopic observations, which showed the similarity between embryonic tissue and cancer, leading to the suggestion that tumors arose from embryo-like cells. The later demonstration in the late nineteenth century of so-called "embryonic remnants" in adult tissues that could become activated in

cancer gave rise to the "embryonal rest" theory of cancer – now understood as the origin of cancer from adult stem cells.

Given their longevity and unique abilities to self-renew and proliferate, it is not surprising that cancers might originate in stem cells. Importantly, the evidence for this is strongest for cancers of the blood and epithelial cells; tissues usually maintained by stem cell replication. The "cancer stem cell" model has recently been supported by a study with another tissue where progenitor cells are the major or only source of cell renewal in the adult, the brain. It was noted that only a subpopulation of brain cancer cells expressing a marker indicating their progenitor cell status were able to generate tumors when implanted into mice. Such xenograft studies have shown similar results for other solid tumors. However, these studies are not without critics. One major confounder is that what we think of as a cancer stem cell population might simply be those cells which make the right kinds of unnatural relationships with cells in the alien environment and avoid immune interactions. When these influences are accounted for by homotypic grafting in very immunocompromised hosts, as many as 20% of cancer cells can give rise to new tumors in the host. It is likely that different cancers will follow different pathways – some will be driven by a very small number of stem cell–like cells, whereas in others a substantial clone of cancer cells will generate new cancer cells as the tumor grows.

A major factor often cited in support of the stem cell origin theory of cancer is the observed similarity between many cancer cells and various embryonic or adult stem cells. However, it is frequently observed that overexpression of many different oncogenes, such as c-*MYC* or *RAS*, may result in a rapid loss of differentiation and re-entry into the cell cycle for various previously differentiated cell types (see Chapter 6). Moreover, various signaling molecules can confer "stemness" on previously differentiated cells by activating EMT programs. In other words, the initiating mutation could equally well occur in a postmitotic differentiated cell as long as such mutations confer or capitalize on the potential of that cell to re-enter the cell cycle. In this scenario, the phenotypic similarities between cancer cells and primitive precursors or stem cells arises not necessarily because this reflects the nature of the cell of origin but rather one of the associated consequences of the initiating oncogenic lesion, whatever the original state of differentiation of the cell involved (Fig. 1.9).

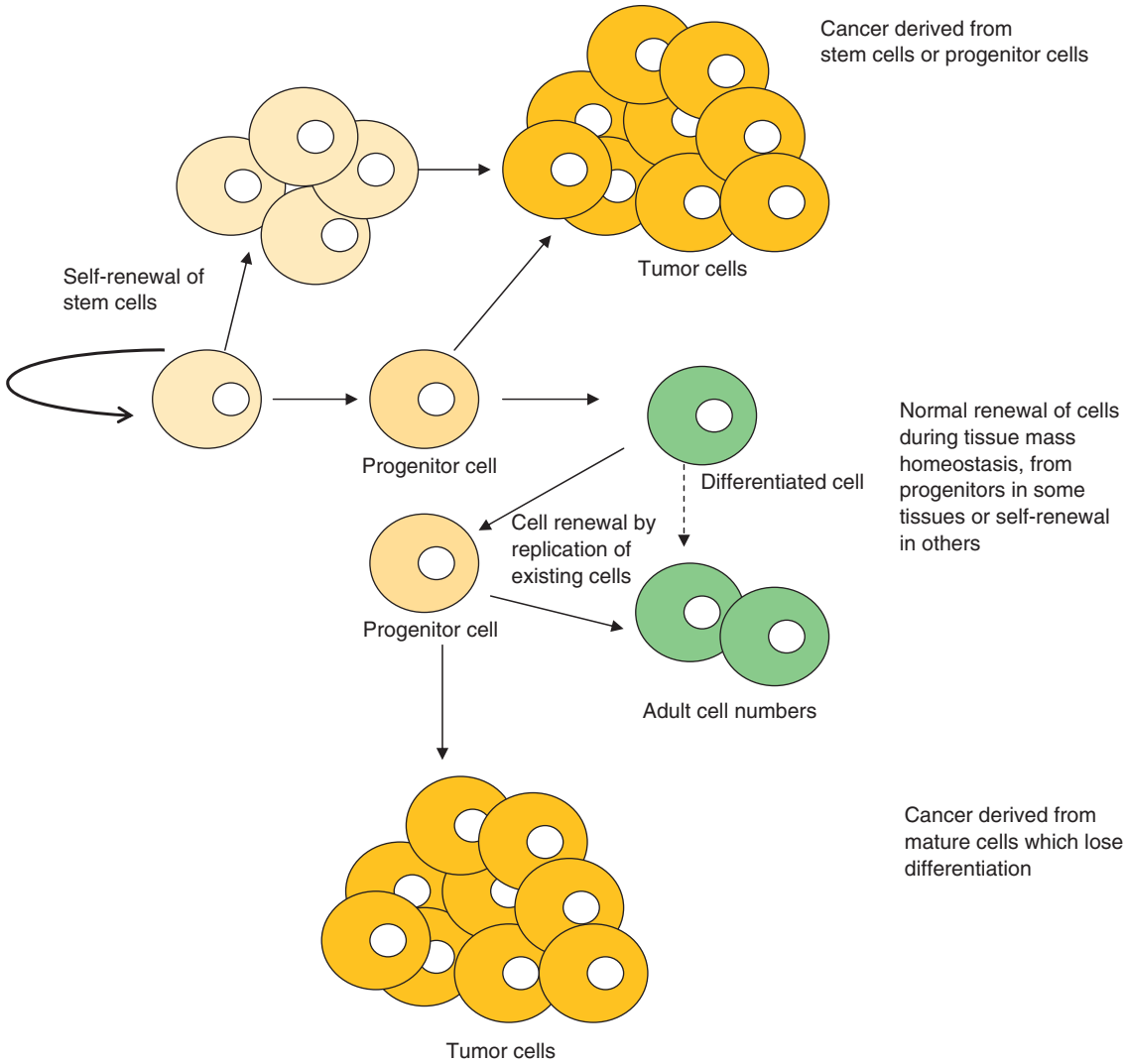


Figure 1.9 Cell of origin of cancer. Cancers probably originate most frequently in progenitor or stem cells, but may also arise from more differentiated cells that lose differentiation as part of the oncogenic process.

If "dedifferentiation" is an inevitable accompaniment of cancer-causing mutations, then the preferential role of stem cells in the initiation of cancer may instead reflect the higher intrinsic rate of replication or their longevity in adult organisms. This is more plausible as it is extremely likely that mutations would occur more frequently during cell division because of the vulnerability during DNA replication. However, this is by no means the only way in which mutations occur (see Chapter 3), and it is not only stem cells that replicate in the adult organism. The observation that "promotion" of an epidermal cancer may be accomplished months or even years after the initial exposure to carcinogen ("initiation") is often taken to imply that the original carcinogenic event occurs in a long-lived epithelial stem cell population. While this is highly likely in skin, where mature cells are continually removed by shedding at the surface, it is equally plausible in other tissues that the original mutation conferred longevity (particularly likely given the repeated observation in mouse models that an antiapoptotic lesion may be among the earliest required mutational events in cancer formation), or that cell turnover of differentiated cells in a given tissue is usually slow (thus, unless the mutation conferred an immediate growth advantage, it would only be passed on to a small number of progeny).

It must be remembered that it is now unarguable that differentiated cells can and do replicate in the adult even under normal physiological circumstances, and in some tissues this may be the sole or major source of new cells. The cellular events during development of liver cancer suggest that cancers may arise from cells at various stages of differentiation in the hepatocyte lineage. Much experimental data support the view that dysregulation of specific genetic pathways, rather than cell of origin, dictates the emergence and phenotype of various cancers, including high-grade glioma and others.

Whatever the actual outcome of these scientific debates, it is clear that treating cancer by inducing its differentiation (differentiation therapy), whatever that may have been in the cell of origin, offers considerable promise. However, it cannot be assumed that this alone will suffice if the cell of origin was differentiated to begin with. Thus, for example, inducing differentiation in c-*MYC*-induced osteosarcomas by transiently inactivating c-*MYC* has recently been shown to alter the epigenetic context surrounding c-*MYC* signaling so as to change this from being procancer to instead becoming proapoptotic (anticancer). Whereas in the case of a c-*MYC*-induced tumor arising from a more differentiated cell type, which in consequence loses differentiation as part of c-*MYC* activation, inducing "redifferentiation" by transient c-*MYC* inactivation does not change the context, and reactivation of c-*MYC* results in further tumor progression. Once again, a general rule holds true, namely, that most things related to cancer are a matter of timing and are also determined by numerous factors including the cell or origin, the mutations accumulated and the cancer environment – together referred to as the molecular "roadmap" of that cancer.

The cancer stem cell and niche

The cell of origin notwithstanding, considerable interest is developing in the existence of CSCs within some if not all tumors. The concept of CSCs was first proposed over 100 years ago, but has only recently hit the mainstream, with the identification of such cells in a variety of human cancers. In fact, there is even interest in the possibility that the particular resistance of CSCs to chemo-radiotherapy may in part explain the failure to cure most metastatic cancers.

Although the clonal nature of cancers is well-established, there are some unanswered questions. Our incomplete understanding is illustrated by studies which suggest that hundreds of thousands of cancer cells may have to be transplanted in order to establish a new tumor from an existing one. Clearly this could simply reflect the chance nature of cell replication and survival, but it is also open to an alternate interpretation, namely that only a small number of cancer cells in the original tumor are capable of initiating a new tumor. When examined at a molecular level, these different possibilities would suggest that in the "chance" model, most if not all cancer cells contain the necessary epigenetic changes needed for tumorigenesis and some get lucky or make the right connections in the new location, whereas the alternative model presupposes that cells in the cancer are very heterogeneous, with only a select minority group of "Über-cancer cells" capable of recapitulating tumorigenesis – the cancer stem cells. These tumor stem cells are a rare population of cells that can reconstitute a new tumor comprising all the cell types present in the original cancer. It is tempting to blame such cells for the formation of metastases and of new tumors following inoculation of cancer cells in a different host organism (xenografts). The CSC hypothesis states that a minority of transformed stem cells, or progenitors with acquired self-renewal properties, are the source of new tumor cells. By implication, such cells are also responsible for the behavior of cancers, such as rate of growth or proliferation, invasion or metastases, and sensitivity to various treatments. Stem cells might be more resistant, for example, to apoptosis induced by chemoradiotherapy when compared to more differentiated cells within the cancer.

Tumor stem cells are akin to adult and embryonic stem cells in that they undergo self-renewal by asymmetric cell division, but they have so far only been unambiguously identified in some hematological cancers, such as acute myeloid leukemia (AML), in which around 1 per million tumor cells may be a tumor stem cell, and in breast cancer, where anywhere up to 2% of tumor cells may exhibit some of these characteristics. The molecular basis of stem cell behavior may prove useful in developing new cancer drugs, and with this in mind the Wnt-signaling pathway and polycomb genes, discussed in later chapters are of particular interest. As with other stem cells, the immediate microenvironment comprising stromal cells (niche) within which such cells exist is just as interesting as the nature of the stem cells themselves.

A chemotherapy-resistant niche

The tumor microenvironment is also a critical determinate of the success of chemotherapy. In a mouse model of Burkitt lymphoma it has been shown that survival of cancer cells in the face of DNA-damaging agents is influenced not just by cell intrinsic factors but also by local secretion of paracrine factors, such IL-6 and Timp-1. These create what the authors describe as a "chemoresistant niche," within which a small number of cancer cells can survive and may be able to repopulate a recurrent cancer.

Targeting the cancer-initiating cells

Cancer stem cells are sometimes referred to as tumor-initiating cells (TICs), which neatly avoids any presuppositions about the nature or origin of the cell. The controversies alluded to earlier notwithstanding, there are numerous points of interest in the

model. Such cells have been proposed in large number of human cancers, though not incontrovertibly by any means, including hematological malignancies and tumors of the breast, prostate, brain, pancreas, head and neck, and colon. Their presence in the tumor may worsen prognosis, may partly account for resistance to conventional chemoradiotherapy, and may provide a specific target within the cancer for new drugs. The latter depends on the identification of unique markers on the cell surface which may allow such TICs to be isolated and studied. However, despite early promise, various markers such as CD133, CD44, and CD166 have not unambiguously defined malignant from normal stem cells in various cancers and moreover do not entirely define the nasty subset of cancer cells within a given tumor. There is much hope that new techniques for concurrent determination of multiple surface markers might address these limitations.

In order to eradicate TICs in cancers we will need to unravel the molecular mechanisms regulating processes such as self-renewal, differentiation, and escape from therapy. Pathways involved in self-renewal and cell fate have been described and include those important in normal stem cells, such as Wnt, Notch, and Hedgehog, but also, tumor suppressor genes such as *PTEN* and *TP53*. Once these pathways are deregulated in TICs they can drive uncontrolled self-renewal, resulting in treatment-resistant cancers, because some rare TICs will survive even if the bulk of the tumor is annihilated. The CSC model implies that curing cancer requires new cancer therapeutics that target and eradicate these CSCs. Reactivation of embryonic/developmental signaling pathways such as Notch, WNT–β-catenin, BMI-1, sonic hedgehog, and EGFR, when combined with drug-resistant mechanisms such as efficient DNA-repair processes, checkpoint regulation and ABC transporter–mediated drug efflux, shown in a variety of TICs may represent new targets for treatment of resistant cancers. The local microenvironment of CSCs, or niche, may also be a target as such location-specific cues may not be critical for other cells.

With this in mind, recent studies have identified the *PTEN* tumor suppressor as a key regulator of TICs in leukemia, brain, and gut, and suggest that drugs such as rapamycin, which targets the PI3K–AKT–mTOR pathway normally suppressed by *PTEN*, might at least in transgenic animals deplete TICs without damaging normal stem cells.

The latent niche

It has been suggested that CSCs may form cell–cell interactions similar to those that have been described for normal stem cells and stem cell niches. Recent studies in the nematode worm have suggested that under some conditions, differentiated cells that do not normally contact stem cells nor act as a niche can promote ectopic self-renewal, proliferation, or survival of competent cells, with which they form aberrant contacts. The authors have described this as a "latent niche." One of the important implications of this mechanism for tumor initiation is that it does not necessarily require genetic changes in the tumor-initiating cell itself. It will be interesting to see if such a mechanism occurs in human cancers.

Cancer is a genetic disease

> Scientists have found the gene for shyness. They would have found it years ago, but it was hiding behind a couple of other genes.
>
> *Jonathan Katz*

With the availability of the reference genome for humans and mouse, the last decade has witnessed an explosion of new knowledge in human genetics. Our understanding of the genetic basis of disease has grown dramatically, with nearly 5000 diseases identified as heritable. Moreover, it is now known that genes contribute to common conditions such as heart disease, diabetes, and many types of cancer.

Currently, more than 1% of all human genes are "cancer genes," of which approximately 90% exhibit somatic mutations in cancer, 20% bear germline mutations that predispose to cancer, and 10% show both somatic and germline mutations. A recently published "census" of cancer genes (see the Sanger Institute website – www.sanger.ac.uk/genetics/CGP/Census/) is dominated by genes that are activated by somatic chromosomal translocations in leukemias, lymphomas, and mesenchymal tumors. Interestingly, the protein kinase domain was the most frequently represented domain encoded by cancer genes, providing support for the development of therapies targeting this domain in cancer, followed by domains involved in DNA binding and transcriptional regulation.

Cancers (and Darwin's finches) evolve by mutation and natural selection

Broadly, cancers arise due to genetic (or epigenetic – see Chapter 11) alterations in three types of genes: oncogenes (see Chapter 6), tumor suppressor genes (see Chapter 7), and caretaker genes, such as DNA-repair genes (see Chapter 10). Combinations of epimutations in these classes produce tumors. Genetic (but most probably not epigenetic) alterations may occur in the germline, resulting in inherited cancer predisposition, or more commonly either occur in somatic cells, giving rise to sporadic tumors. The first somatic epimutation in an oncogene or tumor suppressor gene that enables clonal expansion may be regarded as the initiating insult. Unfortunately, in the vast majority of human cancers this key early step is not known. Tumors progress through the acquisition of further somatic epimutations, which allow further rounds of clonal expansion. Broadly, therefore, tumor cells evolve, with those cells with a growth advantage selected for at each mutational event. Individuals with an inherited abnormality in any of these genes are cancer-prone presumably because they are one step ahead of those without such germline abnormalities.

Blame the parents – inherited single gene defects and susceptibility to cancer

> Children begin by loving their parents; after a time they judge them; rarely, if ever, do they forgive them.
>
> *Oscar Wilde*

Most cancers are not the result of hereditary high-penetrance mutations. In those cancers where inherited mutations are an important contributor they often involve inactivation or silencing of a "caretaker" or tumor suppressor gene. Inherited forms of cancer represent perhaps about 5–10% of all cancers and include two rare inherited cancers, studies of which have resulted in disproportionately spectacular insights into cell and cancer biology in general: a childhood eye cancer known as retinoblastoma (caused by loss of the *RB* tumor suppressor) and

the Li–Fraumeni syndrome (caused by loss of the *p53* tumor suppressor), in which children and young adults of the family develop an assortment of cancers, including sarcomas, brain tumors, acute leukemia, and breast cancer.

More recently, gene mutations associated with common cancers, including colon cancer and breast cancer have been identified. The familial adenomatous polyposis coli gene (*APC*) has been identified as a cause of inherited precancerous polyps, and a contributor to colon cancers. Another inherited form of colorectal cancer, Lynch syndrome, is caused by loss of mismatch repair genes. Possibly the most clinically important hereditary cause of cancer involves mutations in the *BRCA1* or *2* genes and predisposes affected women to both breast and ovarian cancers. It is estimated that as many as 1 in 300 women may carry inherited mutations of breast cancer susceptibility genes. People who inherit cancer genes are more likely to develop cancer at a young age because the predisposing gene damage is present throughout their lives. Recently, a further ovarian cancer susceptibility gene, *RAD51*, has been identified, which is also involved in the DNA-damage response.

Loss of heterozygosity and comparative genome hybridization

Deletion of genetic material is a very common event in human cancer. Indeed, it is the most frequently observed genetic abnormality in solid tumors. There are several mechanisms through which a somatic cell, with an inherited mutated gene allele, can lose the normal gene copy and become vulnerable to cancer (Fig. 1.10, also see Chapter 10). These mechanisms may result in what has been described as loss of heterozygosity (LOH). LOH can occur by deletion of the normal allele, deletion of part of or the entire chromosome (referred to as aneuploidy), possibly followed by duplication of the chromosome containing the mutated allele, or by mitotic recombination (crossing over) with genetic recombination in mitosis (it is a normal part of meiosis). Thus, a particular chromosomal region might be found in 0, 1, 2, or many copies, whereas the similar region in normal cells always have two copies. These extreme genetic aberrations in cancer cells (loss or gain of chromosomal regions) may be readily detectable during cytological examination and such abnormalities can form the basis of diagnostic and prognostic decisions.

Haploinsufficiency

Alfred Knudson's two-hit model of tumor suppressor genes, first proposed in 1971, supposes that two mutations are required to cause a tumor, one occurring in each of the two alleles of the gene (see Chapter 7). Recently, however, tumor suppressors that do not conform to this standard definition have been described, including genes requiring inactivation of only one allele (also referred to as "haploinsufficient"), and genes inactivated by epigenetic silencing (see Chapters 7 and 11).

Blame everyone – complex polygenic mechanisms and inherited susceptibility to cancer

What remains to be uncovered is how low-penetrance genetic variants (polymorphisms) contribute to the risk of developing so-called "sporadic cancers." Polymorphism refers to a gene that exists in more than one version (allele), where the rare allele can be found in more than 2% of the population. The term broadly encompasses any of the many types of variations in DNA sequence found within a given population. Specific subtypes of polymorphisms include mutations, point mutations, and single-nucleotide polymorphisms (SNPs) (see Chapter 10). Although this is an oversimplification, polymorphisms may be regarded as having less dramatic or overt functional effects than mutations.

Although we are a long way from describing variations in these multiple potential gene alleles, we know that polymorphisms contribute to response to carcinogens, variations in drug responses,

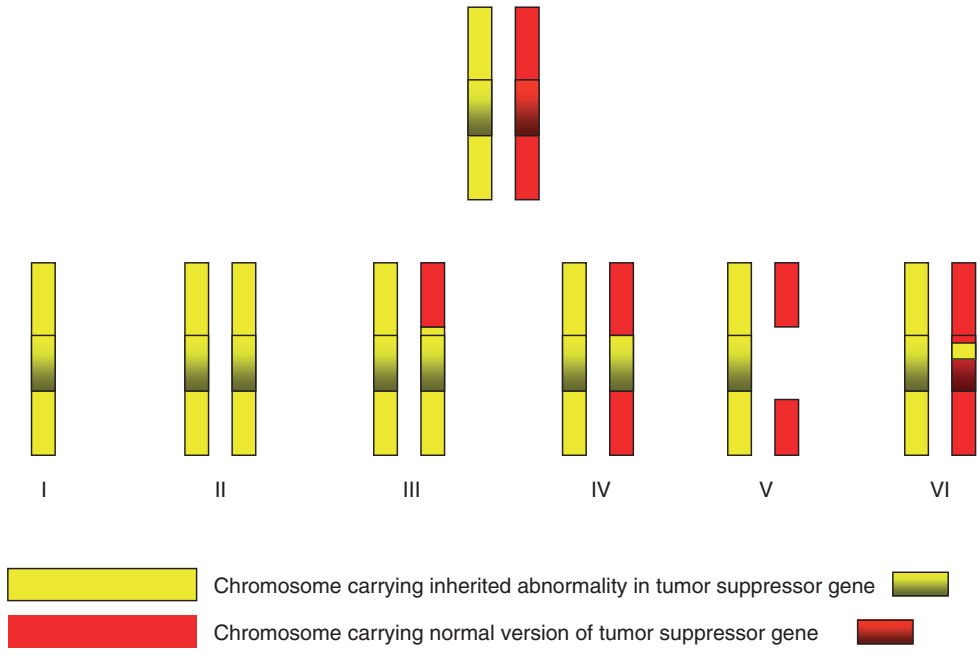


Figure 1.10 Loss of heterozygosity through various genetic events for a tumor suppressor gene. I, Nondisjunction; II, nondisjunction and reduplication; III, mitotic recombination; IV, gene conversion; V, gene deletion; VI, point mutation.

and undoubtedly to many other aspects of cancer. Recently, much interest has been sparked by the identification of polymorphisms, which may contribute to the risk of lung cancer by influencing the susceptibility to carcinogens in tobacco smoke.

An apology to Jean-Baptiste Lamarck: cancer is an epigenetic disease (but you were wrong about giraffes)

Epigenetic information is not contained within the DNA sequence itself, but is transmitted from one cell to all its descendants. Such a control is referred to as "epigenetic," as the DNA sequence is not altered. This is a major potential flaw inherent in attempts to understand diseases by sequencing genomes, as these epigenetic factors will be missed. Some altered gene expressions may be driven by environmental factors such as nutrient levels or hypoxia, and others by means of changes in, for example, methylation of genes. Such changes, as mentioned, do not alter the DNA sequence and yet can be passed on to subsequent generations of cells. But before we all rush out and put parents on the rack to produce the next generation of basketball players, there is as yet little real evidence that such epigenetic factors can be inherited through the germline, as it is generally accepted that most epigenetic information is wiped clean in the germ cells. The closest we get to Lamarck's view of giraffe necks is that the fetus may be conditioned by the intrauterine environment, likely by epigenetic effects. Although this is an example of early environmental conditioning of the individual, there is as yet no evidence that this can affect subsequent generations and thus be truly hereditary.

The importance of epigenetic factors in cancer was first articulated by Feinberg and Vogelstein, who noted generalized hypomethylation of DNA in tumor samples (see Chapter 11). Although the focus of attention is now more on the selective hypermethylation of certain genes such as tumor suppressors, these studies were of crucial importance. Many key genes may be silenced by epigenetic changes during successive cell differentiation stages during development, and two epigenetic events in particular have been associated with transcriptional silencing in cancer cells: methylation of CpG islands in gene promoter regions and changes in chromatin conformation involving histone acetylation. Genes known to be epigenetically silenced in cancers include more than half of all known tumor suppressors, with much data in particular available for *p53* and *PTEN*, and the *MLH1* mismatch repair gene, silencing of which can cause genetic instability thus linking epigenetic and genetic factors. Studies in the Min mouse (*APC*-defective mutation) revealed that reducing DNA methylation with an inhibitor of a key enzyme, DNA methyltransferase (DNMT), reduced intestinal polyp formation directly, establishing the key role of epigenetic factors and tumorigenesis.

Loss of imprinting (LOI) – the silencing of active imprinted genes or the activation of silent imprinted genes – is frequently observed in human cancers and is responsible for overexpression of the gene encoding insulin-like growth factor (IGF)-2 in the pathogenesis of Wilms tumor, in Beckwith–Weidemann syndrome, and in some epithelial cancers, including colon cancer.

"So it isn't really junk after all." Noncoding DNA

Having long been regarded as largely junk, it now turns out that the large amount of DNA that does not actually encode instructions for making a specific protein actually contains important regions involved in regulating gene expression, DNA structure, and cell fate. Some of the noncoding DNA has long been known to contain key regulatory elements for the gene, such as gene promoters, which control gene expression. Perhaps surprisingly, most of the eukaryotic genome is actually transcribed, resulting in a confusing jumble of RNA transcripts that include tens of thousands of microRNAs (miRNAs), long noncoding RNAs, and others with little or no protein-coding capacity. Small RNAs (see Chapter 11) can silence various genes, in part by forming dsRNAs which target mRNAs for destruction.

Most long noncoding RNAs remain uncharacterized but many are likely to represent more than just transcriptional "noise." Some are already known to be differentially expressed amongst differing cell types and conditions and to be localized to specific subcellular compartments. The potential role played in cellular function is far from clear, but might include processing to yield small RNAs; in some cases noncoding RNA transcription itself may affect the expression of adjacent genes and in other cases noncoding RNAs may function in a similar way to proteins and directly influence activity or localization of proteins.

The last few years have seen a huge increase in the amount of information available about the critical role played by miRNAs in posttranslational regulation of gene expression. miRNAs are short, single-stranded RNAs, typically in the size range 19–25 nucleotides. Essentially all cell biological processes are influenced in some way by miRNA, because most if not all signaling pathways are in some way regulated by miRNAs as well as other factors. In cancer, those many miRNAs which can act as oncogenes or tumor suppressors are collectively referred to as "oncomirs." Distinct clusters have distinct functions, and to give you some idea of how complex these regulatory mechanisms are take a look at Fig. 6.8 in Chapter 6, showing the relationship between one transcription factor, c-Myc, and miRNAs. Oncomirs can influence essentially all cellular processes altered during tumorigenesis, and many specific miRNAs with central roles have been identified. These include the mir-17–92, a polycistronic miRNA cluster that contains multiple miRNA components, also known as oncomir-1, which is amplified in several human B-cell lymphomas and can promote proliferation and survival, inhibit differentiation, and increase angiogenesis. Overexpression of miRNAs LIN28 and LIN28B is found in many human cancers and is associated with repression of Let-7 family miRNAs. In turn, loss of Let-7 releases inhibition on targets such as *HMGA2*, *KRAS*, and c-*MYC*, which drive tumorigenesis. Other miRNAs are functionally important targets of *p53* while others regulate the activity and function of *p53*. Other tumor suppressors are also regulated by miRNA, including *PTEN* (see Chapter 7).

Mutations can result in activation of oncomirs. Chronic lymphocytic leukemia (CLL) is typified by chromosomal deletions on 13q, 17p, and 11q, sites at or near the miR-15a/miR-16-1 cluster, p53, and miR-34b/miR-34c clusters, respectively. A miRNA/TP53 feedback loop is involved in CLL pathogenesis and outcome.

Biomarkers in diagnosis and subclassification of cancers and miRNAs can now be detected and measured in serum. The RNA interference mechanism is being used in new therapies with siRNAs, miRNA analogs and antagonists of miRNAs (antagomirs). See Chapter 16.

The cancer "roadmap" – What kinds of genes are epimutated in cancer?

Broadly, three classes of genes are involved in cancer:

- **Oncogenes** – These are usually variants of normal genes that are involved in promoting behaviors such as replication that are

essential drivers of cancer. Unlike their normal cellular counterparts, the proto-oncogenes (a term which rather underplays the important role played in normal cell growth/expansion and rather erroneously conveys the impression that their role is to wait around until they go bad and cause cancer), oncogenes are either abnormally activated or overexpressed versions that can drive aberrant growth in the absence of normal regulatory controls. Not surprisingly, most oncogenes are related to growth factors or more usually their receptors, downstream signaling molecules activated by them, or ultimately the nuclear targets of such signaling pathways and the drivers of the cell-cycle machinery.

- **Tumor suppressors** – Conversely, these normally act to restrain the oncogene signaling described briefly above either by acting as restraints of growth factor signaling or in general ways as guardians of cell stress, DNA damage, or abnormal oncogene-driven growth, to which they respond by promoting apoptosis or senescence or blocking cell-cycle progression. The tumor suppressors must be inactivated in order for cancers to develop. As genes such as *p53* appear in evolution before cancer was likely to have posed any problems to the organism, it is believed that the original role, at least of this tumor suppressor, was something else. In fact, recent studies suggest that *p53* may play a normal physiological function in meiotic recombination.
- **Caretaker genes** – These are involved in sensing and repairing DNA damage. They include the important mismatch repair genes, which may be damaged as a relatively early event in some cancers such as those of the colon, thereby accelerating the development of mutations that activate oncogenes or inactivate tumor suppressors.

It has been estimated that up to seven rate-limiting genetic or epigenetic events are needed for the development of common human epithelial cancers, and these may be ordered in multiple different combinations depending on which particular tissue or cell-specific "anticancer" barriers need to be circumvented and because there may be a number of different effective "routes" available for getting around any given obstruction. And as those of us with satellite navigation systems are only too aware, many of these are far from direct. It should also be borne in mind that the actual number of "mission critical" epimutations needed to initiate cancer may differ depending on which cancer we are considering and which genes are deregulated. Thus, several studies suggest that fewer mutations may be needed if one of the lesions results in persistent or sustained deregulation of activity of the oncoprotein c-MYC (see Box 1.4). Figure 1.11 shows how MYC can cooperate with RAS in tumorigenesis.

Importantly, many key molecular contributors to cancer progression may not themselves be deregulated at the gene level. Thus, downstream signaling proteins may become upregulated because of alterations upstream in growth factor signaling genes, altered catabolism, genes inactivated by epigenetic factors, protein expression altered by enzyme activity, degradation, chaperones,

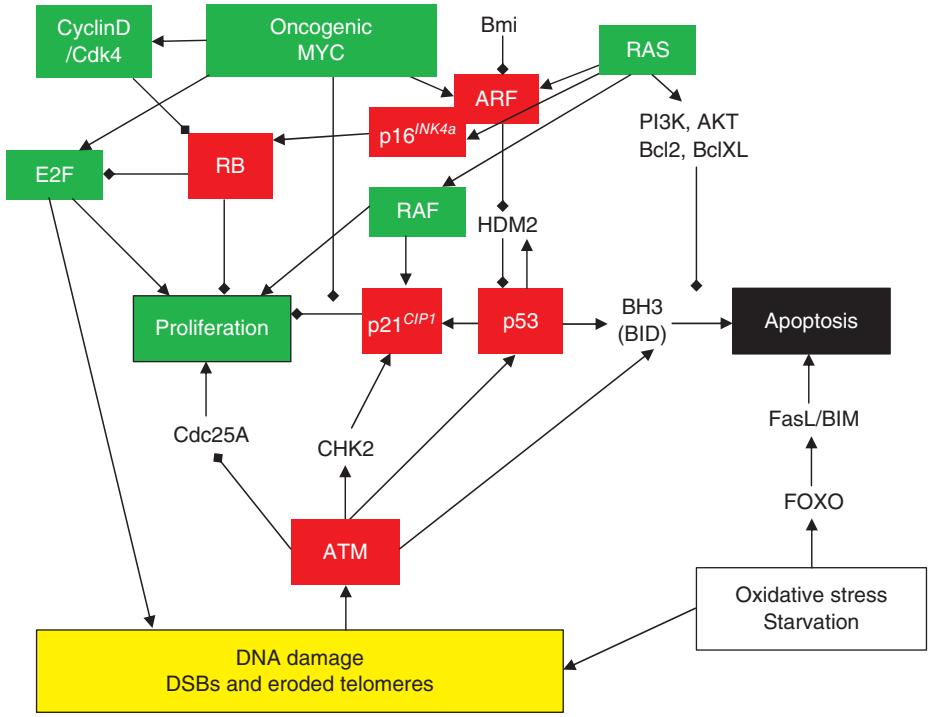


Figure 1.11 Linkage between signaling regulating replication, DNA damage, apoptosis, and growth arrest. Several links exist between mitogenic signaling and that regulating growth arrest and apoptosis. Moreover, DNA damage response pathways may be involved in linking oncogenic cell cycles with growth arrest and apoptosis. Activation of RAS and c-MYC (MYC) via growth factor signaling results in potential engagement of both replication and growth but also of apoptosis and possibly growth arrest. If either MYC or RAS levels are excessive (as might occur during oncogenesis) or other proapoptotic signals are received, then the balance may be tipped away from replication. Oncogenic RAS can promote senescence through either $p16^{INK4a}$ or ARF, which activate the RB or p53 pathways, respectively. Intriguingly, MYC may activate apoptosis through activation of ARF, possibly at least in part via DNA damage responses. Although it remains unclear as to how the cell can distinguish between a normal cell cycle and an aberrant "cancer cell cycle," one possibility is that the latter may be more likely to result in DNA damage. Apoptosis may be blocked by RAS activation of the PI3K and AKT pathways. DSB, double-strand break.

etc. Again, it should be noted that events contributing to cancer are not restricted to the cancer cells. Thus, for example, expression of key cancer-contributing proteins in the cancer cell, such as NF-κB in hepatocytes, may be upregulated through changes in expression of TNF-α in neighboring stromal inflammatory cells.

Viruses and the beginnings of cancer biology

The identification of the genetic mechanisms of transformation owes much to the study of transforming viruses, in which the transforming effect could be attributed to specific oncogenes. DNA viruses express proteins analogous to key proliferation factors that substitute for or replace the function of the cellular factors. In contrast, the oncogenes of RNA retroviruses are derived from the hijacking of critical cellular regulatory genes with the addition of gain-of-function mutations (see Chapters 3 and 6). In fact, many normal cellular genes involved in growth were first identified as viral oncogenes, with the normal cellular counterparts or proto-oncogenes discovered subsequently.

Knowledge gained about DNA tumor viruses and the molecular biology of viral transformation has played a major role in furthering understanding of oncogene and tumor suppressor function and in the development of cancer biology in general, although the actual contribution of viruses to the formation of most human cancers is by comparison rather modest. The studies of SV40 large T antigen and HPV E6/E7 proteins, together with studies of the familial cancers, have proved critical in understanding the importance of the *RB* and *p53* tumor suppressor genes. This is one example of how several fields of study often converge in science to help illuminate a key process (see Chapter 3).

Michael Bishop and Harold Varmus won the Nobel Prize for Medicine in 1989 for their work in showing that the chicken Rous sarcoma virus (RSV) carried an oncogene called v-*src*, a version of a normal chicken gene called c-*src* but without introns, which the virus had hijacked from a chicken host some time during its evolution. This study transcended the identification of a cause of a chicken cancer when it was subsequently shown that many other retroviruses contained oncogenes that had important normal cellular counterparts involved in growth signaling, many of which were discovered in this way.

The role of infection in human cancers has become much better understood in the last decade or so. We now have active vaccination programs in many countries to prevent infection of women with cervical cancer-causing HPV, for example. We have also identified several other less common cancers in which viruses may be important and have found that infection with the bacteria *Helicobacter pylori* causes gastric inflammation and ulcers and contributes to gastric cancer.

Hens and teeth or bears and woods? The hens have it – cancer is rare

> Adversity has the effect of eliciting talents, which in prosperous circumstances would have lain dormant.
>
> *Horace*

Given the evolutionary nature of cancer, it is perhaps surprising that three lifetimes are required to generate an effective cancer cell. The mutation rate has been estimated at 1 in 2×10^7 per gene cell division. Given, that there are around 10^{14} target cells in the average adult human, with a myriad of potential target genes involved in regulation of cell expansion, and that the chances of further mutations are greatly increased by clonal expansion of those cells carrying the initial lesion, highly effective innate barriers to cancer must exist. Some of these barriers are now well described and include the coupling of oncogenic proliferative signals to those which induce apoptosis, senescence, or differentiation and the tumor suppressor pathways involving *p53* and *RB*. Large, long-lived animals like humans have a large potential somatic mutational load. It has been estimated that point mutations resulting in activation of *RAS* occur in thousands of cells daily in the average human. As the vast majority of these do not result in neoplasia, it is assumed that the usual outcome of such mutations is apoptosis, differentiation, or growth arrest. It should also be remembered that epithelia, such as gut, have the unique advantage of being able to shed potential cancer cells from the surface into the outside world. Once estranged from their usual nurturing environment, they undergo a form of apoptosis (anoikis) before ending life in the bath, lavatory, or waste disposal. In fact, the perceived ubiquity of cancer in humans is simply a product of the truly mind-boggling numbers of cells in our bodies and the fact that they have to divide so many times during our three score and ten years. If, however, these mechanisms are disabled then cancer may become inevitable.

The barriers to cancer

> Your silence gives consent.
>
> *Plato*

The tumor suppressors

Two key pathways, those involving the tumor suppressors p53 and RB, are among the most critical barriers to cancer development. Not surprisingly, the p53 and RB pathways are frequently inactivated in human tumors and may be disrupted at different points. Thus, genetically, the RB pathway (cyclin D, CDK4, $p16^{INK4A}$, RB), a critical determinate of the G_1/S transition in the replication cell cycle, acts as one "critical target" in cancer cells, but the mechanism of disruption varies according to tissue. Thus, for example, cyclin D is overexpressed by amplification in breast cancer and by translocation in parathyroid cancer; CDK4 is mutated or overexpressed in melanoma; $p16^{INK4A}$ is inactivated by deletion or silencing in melanoma and pancreatic cancer; RB expression is lost by mutation or deletion in retinoblastoma and soft tissue sarcomas. Such patterns may not be random. Specific associations of events are seen within individual tumors, and these presumably reflect the evolution of the tumors along particular pathways.

The p53 tumor suppressor protein is a major component of the natural defenses against cancer. The p53 protein acts by arresting the cell cycle and promoting apoptosis (programmed cell death) in response to DNA damage, hypoxia, or unscheduled activation of oncogenes such as c-*MYC*. The *p53* gene is altered in more than half of all human cancers and, because of its role in mediating growth arrest or apoptosis in response to DNA damage, referred to as genotoxic stress, has been termed the "guardian of the genome." However, given the equally important (and from recent studies, controversially the more important) tumor suppressive

role of p53 activation in response to inappropriate oncogene activation, referred to as "oncogenic stress," this term is somewhat underrepresentative. It might be more accurate to view p53 as the universal overseer of cell stress – a kind of intracellular barman. Thus, recent findings that p53 may also shut down key metabolic processes that allow aerobic glycolysis (the Warburg effect) and the pentose shunt support this view. Mediators and regulators of p53 activities are also targeted in cancer, and inactivation of $p21^{CIP1}$ or ARF or activation of MDM2 (an inhibitor of p53) are all observed in cancers.

Over the last decade, numerous links between the p53 and RB tumor suppressor pathways have been identified, including regulation of the G_1/S transition and its checkpoints. This has highlighted the crucial role of the E2F transcription factor family in these pathways. Virtually all human tumors deregulate either the RB or p53 pathway or both. Many other tumor suppressors are known and are discussed in Chapter 7.

One area that has greatly excited the research community and, incidentally, the pharmaceutical and biotechnology sectors in recent years, has been the unveiling of the crucial role played by noncoding DNA and miRNAs in regulation of gene expression, and in particular how this gets derailed in cancer. Thus we now know of miRNAs which contribute to oncogene activity, to tumor suppressor pathways, and even to regulation of CSCs and EMT. It is extremely likely that miRNAs will be found to contribute to the regulation of essentially everything over the next few years by providing another level at which the activity of genes is controlled.

Avoiding suicidal urges

> When God desires to destroy a thing, he entrusts its destruction to the thing itself. Every bad institution of this world ends by suicide.
>
> *Victor Hugo*

In 1972, John Kerr, Andrew Wyllie, and Alistair Currie published a description of an unusual form of cell death distinctly different from necrosis, which they termed "apoptosis." This is now one of the most published areas of biology (see Chapter 8). Robert Horvitz who, along with Sir John Sulston, was awarded the Nobel Prize for his work on apoptosis has rather succinctly summarized the three stages of apoptosis as follows: "First, killing the cell, then getting rid of the body and then destroying the evidence."

Perhaps the single most critical barrier against cancer is the "selfless" suicide (apoptosis) of a potential cancer cell, which, either because it has been unable to repair damaged DNA or because it is being inappropriately pushed into the cell cycle, disassembles and repackages itself as an energy-giving snack for its neighbors, rather than pose a threat to the whole organism. Apoptosis offers several distinct advantages to the organism, not least of which is a relative absence of inflammation (which might well result if the body had been required to "murder" the potential cancer cell – necrosis). Such an absence of collateral damage during apoptotic death is largely because of the ability of neighboring cells and phagocytes to swiftly recognize and cannibalize the apoptotic cell (usually before it has actually "died"). Moreover, when operating correctly, this also prevents the release of viruses or harmful cellular contents into the environment, instead seamlessly passing them from the apoptotic cell to another cell where they can be neutralized. Arguably, the ability to undergo apoptosis is one of the major hallmarks of moving from a unicellular to being part of a multicellular organism, where "social responsibility" among constituent cells becomes paramount for the survival of the whole organism.

Cells are continually receiving and integrating a variety of both positive and negative growth signals. One intriguing result of much research over the last 20 years has been the appreciation that cells seem only too willing to commit suicide. In fact, cells require continuous signals from neighboring cells in order to survive. Loss of these normal "survival" signals or an increase in negative growth signals will tip the balance and a cell will undergo apoptosis. Two major pathways of apoptosis are known: one is **intrinsic** and is integrated by a variety of signals operating at the mitochondria and the other **extrinsic**, triggered by activation of cell surface receptors such as FAS or TNF receptor. Both pathways eventually activate cascades of caspases, expressed as inactive zymogens, which when activated in cells destined to undergo apoptosis execute the necessary steps for apoptosis. However, the initiating caspases (apical caspases) differ – the intrinsic pathway commences with activation of caspase-9, while the extrinsic starts with caspase-8.

In cancer, the intrinsic pathway of apoptosis may be triggered by "sensors" that determine the presence of irreparably damaged DNA or inappropriate attempts to engage the cell-cycle machinery, which in turn may be modulated by external signals, which either prevent or provoke apoptosis. In general, these mechanisms are largely integrated at the mitochondria. Although, the body rarely "murders" would-be cancer cells it can certainly drive these cells to suicide. The extrinsic pathway is utilized by the immune system to engage the apoptotic machinery via surface "death receptors." These death receptors, which include those for TNF and FASL, respond to some secreted inflammatory cytokines and to some populations of T cells. The pathways activated by these receptors include those able to trigger caspase cascades independently of the mitochondria.

Apoptosis can also be executed by caspase-independent death effectors, such as apoptosis-inducing factor (AIF), endonuclease G, and a serine protease (Omi/HtrA2), released from mitochondria during permeabilization of the outer membrane. It is worth noting that many of these proteins have important or even essential roles in cellular processes unrelated to cell death. AIF and Omi/Htra2 are involved in redox metabolism and/or mitochondrial biogenesis; caspase activation is essential in some cells for terminal differentiation, lipid metabolism, inflammatory responses, and proliferation. This has important ramifications, as it implies that certain key parts of the apoptotic response could not be ablated therapeutically without impeding normal cellular functions, unless drugs can be designed to target only the lethal (and not vital) role of these proteins.

Necrosis is the form of death once thought to be the major if not only cause of death of cells. Apoptosis is a friendly form of cell elimination as collateral damage is slight and free of inflammatory consequences, largely because the corpses are removed fast and intact. Moreover, gorging on apoptotic corpses leaves macrophages sated and quiescent and may even sooth a previously activated and inflammatory cell. Sadly, necrosis does not share these soothing qualities and involves the release of proinflammatory molecules, which can be extremely damaging particularly if the necrotic cell is loaded with destructive agents, such as macrophages and neutrophils. Further inflammatory cells are recruited and healing may be delayed, potentially contributing to

chronic inflammation if either the cause of the necrosis is not removed or phagocytosis is impeded. With this in mind, it is worth noting that in some types of chronic inflammation apoptosis may end in necrosis if the phagocytosis of the apoptotic corpses is delayed – a situation that arises in the presence of high levels of oxidized LDL cholesterol (ox-LDL), due to competition for scavenger receptor-mediated uptake in macrophages.

Chronic inflammation is a contributor to many epithelial cancers and underpins the cancer association between ulcerative colitis and colon cancer and the origin of some gastric cancers, esophageal cancers and probably most non-small-cell lung cancers (NSCLCs). Why? Because inflammatory proteins such as IL-1 may promote proliferation and angiogenesis; inflammatory cells can facilitate spread by producing matrix-degrading enzymes and through the formation of a cancer-supporting stroma. This begs the question as to whether a build-up of ox-LDL, characteristic of adverse lifestyle, obesity, and diabetes may also contribute to cancer and whether statin drugs might be protective.

Other forms of death

Apoptosis and necrosis are not the only forms of cell death described; others include anoikis, endoplasmic reticulum stress, and autophagy. **Autophagy** is essentially self-cannibalization, in which cells collect some of their own organelles and cytoplasm and then proceed to digest them within lysosomes, subsequently using the breakdown products to generate energy and construct new proteins. We have all have seen movies in which the protagonists survive bitter cold by burning the furniture – well this is the cellular equivalent. The cell survives adversity and also gets to replace old and damaged organelles, such as ribosomes and mitochondria. Although autophagy may help the organism survive adverse conditions and may restrict degenerative diseases, it can also be exploited by cancer cells, which may use autophagy to survive in preangiogenic conditions until nutrient delivery can be secured. Autophagy can be stimulated by most forms of cellular stress, including nutrient or growth factor deprivation, hypoxia, DNA damage, and damaged organelles, and is integrated with other cellular stress responses by multifunctional stress-signaling molecules such as p53 and mTOR. Thus, autophagy may be triggered by downregulation of key metabolic sensor signals such as mTOR and can be regulated by p53 through a new family of proteins known as damage-regulated autophagy modulators (DRAM). Beclin, a member of the BH3-only family, triggers autophagy and provides some interconnection with apoptosis. Autophagy appears to be another potential barrier to tumorigenesis that must be overcome. However, autophagy may also be a contributory factor to tumor cell dormancy, which, if released, could give rise to recurrence after therapy.

Anoikis is a form of homicidal homesickness that specifically refers to a variant of apoptosis noted in cells that have become estranged from their ancestral homelands.

Avoiding senescence

In 1961, Leonard Hayflick and Paul Moorhead found that many human cells, such as fibroblasts, had a limited capacity to replicate themselves in culture. In fact, they observed that cells can undergo between 40 and 60 cell divisions, but then can divide no more, a process described as senescence, or they die. This number is often referred to as the "Hayflick limit." Cellular senescence is associated with aging and longevity and has also been termed "replicative senescence." The Hayflick limit for dividing cells may in part be determined by the length of telomeres, which are noncoding regions at the tips of chromosomes (see Chapter 9). Cell division requires the duplication of chromosomes, but each time a chromosome reproduces itself, it loses a part of the telomere (telomere attrition). Once a cell's telomeres reach a critically short length, the cell can no longer replicate its chromosomes and thus will stop dividing. Such cells are termed "senescent." Cells taken from older humans divide fewer times before this occurs, as the "chromosome clock" has been ticking throughout adult life (*vide infra* – stem cells appear less bound by these restrictions).

A key feature of cancer cells is that they have found the means to avoid death and senescence, a form of cellular immortalization. In testimony to their remarkable longevity, cancer cell lines are routinely distributed, cultured, and studied in laboratories across the globe. In most cases these are cells derived from a human or animal cancer that continue to divide under appropriate cell culture conditions with scant regard for the Hayflick limit; because they essentially never stop dividing, such cell lines constitute a limitless supply of cancer cells for laboratory use.

In a spectacular illustration of the resilience and fecundity of cancer cells, the famous HeLa cell line has been dividing ceaselessly since the progenitors were first harvested from a cervical tumor biopsy of a single patient, Henrietta Lacks, in 1951. This was the first human cell line and, in large part because of the generosity of George Otto Gey, who made these cells available to any interested researchers, it has quietly revolutionized cell biology. Interestingly, much as the original cells would have done during the life of the patient, HeLa cells growing in culture plates in different laboratories continue to evolve and several variant strains are now known. What they share is the ability to keep dividing as long as they are appropriately nourished and kept free of infection. Although, clearly, cancer cells do die through hypoxia, extensive DNA damage/chromosomal instability, etc. Cellular senescence may have evolved as one mechanism to avoid cancer, which clearly increases in frequency with aging. Several studies have shown that the induction of cellular senescence can inhibit particular cancers.

Importantly, the majority of cancer cells seem able to avoid telomere attrition (shortening). Thus, expression of the telomere-stabilizing enzyme telomerase is induced in tumors and effectively allows cancer cells to rewind their odometer and enjoy unrestrained replication. However, this situation is not as straightforward as it might at first appear. First, inactivating telomerase in some models of viral oncogene-induced cancers does not impede tumorigenesis or growth potential, suggesting that alternative methods for telomere maintenance are also important. Moreover, in other cancer models, where p53 is inactivated, telomere shortening, instead of promoting apoptosis or senescence, may instead lead to a more genetically unstable cancer as chromosome rearrangements are favored.

Oncogene-induced senescence

As if this were not already complex enough, senescence can also be triggered by activation of various signaling pathways (see Chapter 9). Long appreciated as a major restraint to replicative potential *in vitro*, several recent studies have now confirmed that oncogene-induced senescence (OIS) is also a key inherent restraint to tumorigenesis (along with apoptosis) *in vivo*. Although the exact signaling pathways most critical for OIS may vary for different cell types and cancers, there are common features and

overlap with activation of DNA-damage responses such as those seen with telomere attrition and variously engagement of either the ARF–p53–$p21^{CIP}$ and/or $p16^{INK4a}$–Rb pathways. What remains unclear is for how long such senescent cells persist before being culled and whether this state is truly and always irreversible.

One intriguing question in biology is why damaged cells under some circumstances undergo growth arrest or senescence rather than apoptosis – they forsake Eros rather than embrace Thanatos.

Oncogenes as tumor suppressors

Studies over the last two decades have revealed another crucial antineoplastic mechanism, namely that many signaling networks promoting cellular replication also possess intrinsic growth-suppressing activities. Under normal growth conditions, such as tissue maintenance and repair, signaling networks are activated in a coordinated fashion by appropriate extracellular signals, which can block the growth-suppressing pathways and the cell replicates and survives. However, inappropriate activation of a potentially powerful replicative signal such as c-*MYC*, for instance by mutation, occurs without activation of those other key collaborative pathways; so instead of unscheduled replication the mutated cell dies by apoptosis, thereby eliminating the risk of further mutations and cancer. This "intrinsic tumor suppressor" activity is manifested by several mitogenic proteins; the resultant apoptosis or growth represents a critical "failsafe" mechanism in the avoidance of cancer. By implication, therefore, the inherent growth-suppressing activities of oncogenes such as c-*MYC* must first be suppressed if cancers are to develop or progress – an example of oncogene cooperation discussed in detail in later chapters.

What is the secret of cancer developme . . . "timing"

The exact role of any given protein may be largely a matter of timing with respect to the stage of a cancer's evolution and likely also the developmental stage of the cell under consideration. Thus, even individual proteins within the cancer cell can exert widely differing effects on phenotype. Mitogenic proteins like c-MYC may prevent the initiation of cancer through their inherent apoptotic activity, but once the cancer cell has acquired the ability to avoid apoptosis, or the environment provides sufficient survival signals, it may instead confer a wide range of cancer-promoting behaviors.

A recent study has shown that brief inactivation of c-MYC was sufficient for the sustained regression of c-MYC-induced invasive osteogenic sarcomas in transgenic mice; subsequent reactivation of c-MYC led to extensive apoptosis rather than restoration of the neoplastic phenotype. Possible explanations for this outcome include changes in epigenetic context that may have occurred within the cell type, that is, between the immature cell in which c-MYC was originally activated and the differentiated cell resulting from subsequent (brief) inactivation of c-MYC. In this tumor model, although c-MYC expression is initiated in immature osteoblasts during embryogenesis, subsequent inactivation of c-MYC in osteogenic sarcoma cells induces differentiation into mature osteocytes. Therefore, reactivation of c-MYC now takes place in a different cellular context and induces apoptosis rather than neoplastic progression.

TGF-β was initially identified in culture media from transformed cells as part of a factor that could produce a transformed phenotype in a nontransformed cell line. The observations that TGF-β1 inhibited the growth of epithelial cells, and that inactivating mutations within the TGF-β1-signaling pathway occurred in many cancers, supported the view of TGF-β1 signaling as a tumor suppressor pathway for early stages of cancer. However, many human carcinomas overexpress TGF-β1 and it is associated with a poor prognosis and metastasis. Similar results pertain to tumor cell lines and animal models. Together, this suggests that TGF-β1 switches from tumor suppressor to oncogene as the context changes, probably due to genetic or epigenetic alterations in tumor cells or stromal cues. Thus, the role of TGF-β1 in cancer is stage-specific.

Location, location, location – the cancer environment: nanny or spartan state

Numerous studies now point to the crucial interplay between the cancer cell and its local and systemic microenvironment. It is often assumed that the body is largely a hostile environment for an incipient cancer, with hostilities beginning upon recognition of the errant cells with the express aim being to kill, contain, or starve them into submission. In this Nietzschean power struggle, immune and inflammatory responses are mobilized to eliminate the cancer cells, stromal cells form an impenetrable barrier to contain the spread of cancer cells, and both blood supply and nutrients are withheld from the growing tumor. By implication, cancers will need to overcome these hostile forces in order to progress. As in ancient Sparta, newborn cancer cells are left exposed to die – and it is worth noting that this was an experience that made any survivors strong and nasty.

However, it now appears that for many cancer cells the new infrastructure of a growing tumor may actually represent a *locus amoenus* – a safe haven and nursery in which they may be cosseted and eventually fledged.

Cancer cells as "cuckoos"

It is entirely possible that the rareness of cancer (at a cellular level) reflects the success of these extrinsic hostile forces as well as of intrinsic tumor suppressors in eliminating the inchoate (rudimentary and not fully formed) tumor cells. However, recent studies have increasingly challenged this heroic view in favor of a more nuanced one that acknowledges the sometimes ambiguous relationship between cancer and noncancer cells. Thus, at least once a tumor has become established, cancer cells find ready allies to their cause and environmental interactions that actively support their expansion and spread and that might even offset suicidal urges (see Chapter 12). In fact, the developing tumor may well be – or at least become with time – a nanny state in which newborn cancer cells want for nothing and are fed, sanitized, and cosseted, perhaps because, like unfledged cuckoos, they are not recognized as different.

Cancers, chronic inflammation, and tissue remodeling

In some circumstances, such an ideal microenvironment may precede the cancer rather than evolve alongside it. Thus, chronic inflammation has long been known to increase risk of many cancers, possibly by increased mitogenesis (and thereby mutagenesis) or through paracrine effects from inflammatory cells. In

fact, cells enlisted to serve in wound healing or inflammatory engagement are allowed *interregnum* privileges denied to their "peacetime" counterparts, including a license to migrate and proliferate. Perhaps not surprisingly, such liberated cells may be peculiarly susceptible to becoming cancer cells. In fact, once corrupted by epimutations they may fail to relinquish the extraordinary freedom they enjoyed, even when calm has been restored – a big headstart to cancer.

However, even in the absence of preceding inflammation, malignant transformation takes place within the context of a dynamically evolving "microenvironment" and is accompanied by fibroblast proliferation and transdifferentiation, extracellular matrix deposition and remodeling, increased matrix metalloproteinase expression and activity, infiltration of immune cells (see Chapter 13), and angiogenesis (see Chapter 14). It is readily appreciated how such a milieu may actively support tumor cell invasion, survival, and growth and this is particularly important in epithelial carcinogenesis (see Chapter 12).

Liaisons dangereuses encourage tumorigenesis

Recent studies in epithelial tumors extend the pernicious repertoire of matrix activities during tumorigenesis beyond that of a supporting role. Thus, matrix cells and others may conspire together to initiate and encourage designate cancer cells to participate in promiscuous behaviors conducive to cancer, including proliferation, EMT and invasion, and may even permanently damage the DNA. Moreover, these permissive changes may extend even to normal epithelial cells. Thus, matrix can trigger production of matrix metalloproteinases (MMPs) and reactive oxygen species (ROS) in epithelial cells and the stiffer, more fibrotic stroma present in tumors when compared to normal connective tissue can provoke activation of Rho family members.

Interactions between cancer cells and other cells in their environment are thus key determinants of tumor progression.

Policemen or agent provocateur – immunocytes in cancer

Interactions between tumor-infiltrating leukocytes and tumor cells are also of key importance given that immune cells might either interfere with tumor progression or actively promote tumor growth. Certainly, context is likely to be a critical factor, when it is considered that many cytokines and inflammatory products may not only act as anticancer barriers but could also support cancer behaviors such as growth and invasion. The roles played by stage of cancer evolution and the ability of cancer cells to resist the negative and yet benefit from the positive aspects of immune responses are now being unravelled.

Despite the existence of tumor-specific immune cells, most tumors appear to have acquired a means to avoid immune attack. In recent years a considerable interest has developed in "immune privilege" (see Chapter 13). Foreign antigens that enter immunologically privileged sites, of which the eye, brain, and testis are examples, can survive for an extended period of time, whereas the same antigens would normally be swiftly eliminated elsewhere. It has been proposed that the tumor microenvironment may become a site of immune privilege, possibly through factors produced by the tumor, which might impair immune surveillance. Immune privilege could provide a "safe haven" for cancer cells. Recent studies in ovarian cancer have suggested that one means of immune privilege is recruitment of regulatory T cells by the tumor. These regulatory T cells can block the activity of those T cells that are reactive to tumor antigens, thereby interfering with tumor-specific T-cell immunity and enabling progression of ovarian cancers *in vivo*. Other possibilities include production by the cancer cells of cytotoxic or inhibitory factors for tumor-reactive T cells, such as galectin-1, TGF-β, or Fas ligand.

Neutrophils may play a role in facilitating the metastatic capabilities of circulating cancer cells, for example those that become trapped in small blood vessels within the lung. Thus, neutrophils may play lifeguard and actually help anchor these cancer cells within the capillary endothelium. Interestly, release of IL-8 by cancer cells may attract the attention and assistance of neutrophils, thereby representing a potential target for drug therapy in cancer.

Location also affects tumorigenesis in other ways. Thus recent studies have started to unravel differences between sites in the way in which key tumor suppressor pathways are activated and regulated. Thus, oncogenic Ras strongly activates the Ink4a/Arf locus, in some cases promoting cell-cycle arrest or senescence. Lung tumors form independently of $p19^{Arf}$, whereas $p19^{Arf}$ must be disabled for formation of sarcomas. These differences in behavior between tissues may in part reflect the action of Polycomb-group complexes, which repress Ink4a/Arf in lung tumors.

Cancer goes agricultural

The field effect

D.P. Slaughter and colleagues first introduced the notion of a "field effect" following studies on oral squamous carcinoma in 1953. They identified the presence of histologically abnormal tissue surrounding the carcinoma. This field effect was proposed to underlie development of multiple primary tumors, in the absence of familial predisposition, in the same tissue and possibly also recurrence locally following treatment. According to the multistep carcinogenesis model of Fearon and Vogelstein, propitious genetic alterations accumulate in a more or less stepwise fashion by natural selection, so that clones emerge sequentially, each with growth advantages over the preceding one and thus evolve eventually into cancer. One implication of this model is that precancerous cells in proximity to the cancer will represent earlier "less successful" clones and will have some, but minus one or more, of the genetic alterations present in the adjacent cancer.

This model is supported by studies in a variety of human cancers, including lung, gut, cervix, and prostate, which show genetic alterations in the vicinity of the cancer. More recently, epigenetic alterations in methylation have been shown to contribute to this field effect in premalignant conditions such as Barrett's esophagus and in colonic mucosa affected by ulcerative colitis, and also in prostate cancer and NSCLC. In one recent study of colorectal cancer, a field effect comprising MGMT (O6-methylguanine DNA-methyltransferase) promoter methylation was shown in normal-looking mucosa 1 cm from the tumor margin and not 10 cm distant.

As we have discussed already, paracrine interactions between epithelial cancer cells and adjacent stroma are important and may in some cases actually boost the tumorigenic potential of the cancer cell. Furthermore, tumor-associated stroma is notably heterogeneous in terms of fibroblast behavior, gene expression and may itself demonstrate increased motility and invasive potential. Recent studies using laser-capture microdissection to examine

the stromal and epithelial compartments of primary breast cancers have shown that the stroma bears mutations and loss of heterozygosity of the tumor suppressor gene *TP53* different to those present in the epithelium. In fact, surprisingly, in more than a quarter of breast cancers the stroma had *TP53* mutation even when none could be demonstrated in the cancer cells. In fact, as there was no overlap in the loss-of-heterozygosity profile between the cancer and the stroma, different pathways of clonal expansion must have been involved.

The intriguing fact that tumor-associated stromal fibroblasts may themselves have oncogenic mutations raises many interesting possibilities. Thus, a common epithelial progenitor cell may have given rise to both the tumor and the associated stromal cells. Such EMT has been shown in generating tumor-associated myofibroblasts, which therefore share a common genetic lineage and carry the same mutations. So what about when the mutations are different and lineage must differ? One possibility is that the cancer microenvironment is mutagenic due to ROS from immunocytes and possibly any carcinogens that contributed to the development of the tumor in the first place. The field effect may also explain these findings, with disease causing epimutations present in both the tumor and surrounding "field."

The seed and the soil: metastatic spread

As tumors progress, cells within them develop the ability, or the inclination, to invade into surrounding normal tissues and through tissue boundaries to form new growths at sites distinct from the primary tumor. The seeding and growth of cancer cells in distant organs is termed "metastasis" and is the ultimate cause of death in around 90% of cancer patients. Metastasis was first described in 1839 by the French gynaecologist Joseph Recamier, and soon thereafter, physicians found that certain cancers were most likely to spread to certain organs. Breast and prostate cancer, for example, move to lymph nodes, bones, lung, and then the liver. Skin cancer tends to spread to the lungs, colon cancer targets the liver, and lung cancer typically moves to the adrenal glands and the brain.

In 1889, Stephen Paget proposed that cancer cells shed from an initial tumor were dispersed randomly throughout the body by the circulatory system. He called these circulating cancer cells "seeds" and proposed that only some seeds fall onto "fertile soil" – organs where they can grow. About 30 years later, a researcher named James Ewing proposed an alternative nonrandom model by which circulating cancer cells become trapped in the first small blood vessels, or capillaries, they encounter and then grow in the surrounding organ.

While much is now known about molecular alterations that contribute to tumorigenesis, the genetic and epigenetic alterations that result in metastatic spread of the disease are less well understood. Although as with initiation and progression of other cancer behaviors it now seems that inherited as well as acquired factors may contribute to the likelihood or not of developing metastases, analogous to the hallmark features of cancer, there are hallmark features specifically related to metastasis. These include the abilities to:

- escape from the primary tumor,
- intravasate into local blood vessels or lymphatics,
- survive within the blood or lymphatic fluid,
- extravasate into a distant tissue,
- proliferate within the new environment (metastatic colonization), and
- evolve in parallel to cells within the primary tumor.

Metastasis conferring mutations, and at least some of the resultant behaviors, are believed to be atavistic. As a result, it is often assumed that the ability to invade or metastasize is a chance byproduct of mutations that were originally selected for because they gave cancer cells in the original tumor locus a growth advantage. Over the last decade the view that occasional cancer cells might elope from the primary tumor and settle down to start a family in some distant site has been challenged. Rather, it appears that many solid cancers may experience the exit of large numbers of cells from their homeland in a mass "Volker Wanderung" to seek pastures new even if few will succeed to establish new colonies.

In fact, millions of tumor cells can be shed into the vasculature daily, so why are so few secondary tumors formed? The general explanation for this has relied on the assumption that a number of additional genetic events had to occur in order for a small subclone of cells to arise with the capabilities to enter, navigate, and exit the vasculature and thence to colonize a distant site. However, some recent studies suggest that genes required for metastatic spread may already be expressed in primary tumors and before any metastatic spread, suggesting that metastatic ability might be preprogrammed in tumors by the initiating oncogenic mutations. One problem with such data is that even though multiple genes were aberrantly expressed in such primary tumors, they may not all have been so in any individual cell (gene expression profiles were generated from mushed up whole tumors and epigenetic factors were not addressed).

In the past decade much has been learned about how cancers metastasize. Key findings have included the observation that cancer cells are subject to growth regulation at the secondary site and moreover the molecular characterization of proteins that can suppress the metastatic phenotype. These proteins are encoded by metastasis suppressor genes (MSGs), defined as genes that suppress *in vivo* metastasis without inhibiting primary tumor growth when transfected into metastatic cell lines and injected into experimental animals. To date, over 20 such MSGs have been identified and may represent novel disease biomarkers as well as therapeutic targets. Among the best described of these are *NM23*, *PEBP1*, *RECK*, *KAI1*, *RHOGD12*, *KISS1*, and *CTGF*.

Key processes required for metastatic spread include migration and invasion of tumor cells, requiring cancer cells to detach from the primary tumor and then travel to secondary sites via the lymphovascular systems. Cancer cells are able to secrete MMPs and alter expression of cell adhesion molecules (see Chapter 12) that facilitate invasion by degrading extracellular matrix and disrupt cell–matrix and cell–cell interactions. Once in the maelstrom of the circulation, cancer cells must survive being buffeted by blood flow shear forces and the full broadside of immune assault. Finally, once entrapped within capillary networks they must find the means to extravasate into the ambient tissue and establish a foothold. Various proteins have been implicated in these processes, including cell adhesion molecules, proteolytic enzymes, and members of the RHO family, including RHO, RAC, and CDC42, that are involved in cytoskeletal organization.

Recent exciting data suggest that invasive and metastatic potential is related to reactivation of general embryonic pathways involved in morphogenesis and might include mutations that deactivate E-cadherin and other cell adhesion molecules, those

that activate transcription factors and signaling molecules such as NF-κB and TWIST, which might promote EMT. EMT, originally described *in vitro* as dedifferentiation of epithelial cells to fibroblastoid, migratory, and more malignant cells, with an accompanying altered mesenchymal gene expression program, correlates well with late-stage tumor progression. Typical phenotypic features of EMT include loss of E-cadherin and acquisition of vimentin immunoreactivity. EMT also occurs during embryonic development and is regulated by a complex network of signaling pathways, including the RAF–MEK–MAPK pathway, PI3K–AKT pathway, NF-κB, and TGF-β. In various animal models systems, metastatic potential strictly correlates with the ability of epithelial tumor cells to undergo EMT. Importantly, it is now likely that EMT may also promote the development of CSCs and may provide a further link between inflammation and cancer.

Other recent studies have now added to the complexity of metastasis biology. As discussed earlier, metastatic tumors can secrete factors into the circulation that prepare a distant site for colonization. More recently, it has also been shown that some nonmetastatic human tumor cells can secrete factors, such as prosaposin, that conversely, in part by inducing thrombospondin-1 expression in fibroblasts, renders the microenvironment in distant tissues resistant to colonization.

A question that is currently of tremendous interest is at what time cancer cells acquire the capabilities to undergo metastatic spread. This is addressed in the next section. As in so many other areas of cancer biology, miRNAs have also been shown to have a profound influence on metastasis. Specific networks of miRNAs have been described which affect tumor metastasis, EMT, and invasion through posttranslational alterations in gene expression and epigenetic changes.

Another underexplored area of research is how cancer cells first gain entry into the systemic circulation by directly intravasating into venous capillaries or indirectly via lymphatics.

Treatments based on the identification of MSGs are available; clinical trials of drugs targeting NM23 as an antimetastatic therapy are in progress, although the challenges inherent in trying to restore missing function are substantial (much easier to try and inhibit an overactive protein than replace a missing one).

Cancer superhighways – blood vessels and lymphatics

The metastatic spread of tumor cells is most often the lethal aspect of cancer and frequently occurs via the lymphatic system. Many tumor types, including breast and prostate cancers and melanoma, first metastasize via lymphatic vessels to regional lymph nodes. The presence of lymph node metastases is associated with poor prognosis, but that the lymphatic system might actively participate in cancer metastasis has only been unravelled recently. In fact, tumor-induced lymphangiogenesis may precede lymph node metastases and might therefore be a novel target for prevention. Lymphangiogenic growth factors, such as VEGF-C and VEGF-D, act on cognate receptors such as VEGFR-3 on the surface of lymphatic endothelial cells to promote lymphangiogenesis and metastases to lymph nodes. Interestingly, recent studies suggest that lymphangiogenic growth factors from the primary tumor can induce lymphangiogenesis in nearby lymph nodes before the arrival of metastasizing tumor cells.

On your bike and turn the lights off before you go

One area of considerable general interest is the role played by light–dark and sleep–wake cycles (diurnal and circadian rhythms) in various aspects of cellular biology. At a whole-animal level, it has long been known that many hormonal processes, arousal/alertness, and mood are strongly influenced by sleep–wake patterns and that under usual circumstances in humans these are inextricably linked to day–night cycles. However, when this goes awry, as in shift work or in those who frequently cross time zones, these sleep–wake and light–dark cycles become disconnected and ill-health may result. At the benign end this may cause transient jet-lag, but recent studies have suggested that in some cases there may be more serious consequences, including an increased risk of cancer.

Normally, diurnal and circadian rhythms and cell proliferation are coupled in humans. Various animal studies have shown that exposing rats and mice to light at night can accelerate cell cycle and this is associated with increased IGF-1R/PDK1 signaling and accelerated tumorigenesis. Perhaps it is time to discard the night light?

Catching cancer

Recent studies have confirmed some long-suspected and intriguing notions about cancer cells, namely, that they might be spread between individuals (i.e. you might be able to "catch" cancer like a cold). It is crucial to note the difference between being infected with a cancer-causing virus from another individual, not at all controversial and well exemplified by HPV infection and cervical cancer, and being infected by another person's cancer cells directly. Thus, it now seems that cancer cells do not necessarily perish along with their host but might carry on through generations by spreading to further individuals. In canine transmissible venereal tumor (CTVT), tumor cells are implanted from one animal into a new host, where a new tumor grows – effectively analogous to a transplanted "graft." This raises interesting questions as to how cancer grafts avoid rejection; in CTVT, tumor cells downregulate expression of major histocompatibility complex (MHC) molecules involved in immune recognition, though in many cases an immune response against the tumor eventually does occur and eradicates the cancer. A similar infectious cancer has been described in Tasmanian devils.

In both these cases the infectious nature of the cancer has been revealed by genotyping the cancer cells from numerous different animals from different geographical areas (at least with CTVT-carrying dogs) and showing that these are more genetically similar to one another than they are to the host cells and less genetically variable than even very inbred dogs are to one another. Although such infectious cancers are yet to be demonstrated in humans it is worth noting that certain types of cancer transmission are known. For example,

- During pregnancy, transplacental transmission of leukemia, lymphoma and melanoma to the fetus has been demonstrated.
- Organ transplants carrying occult cancer cells have been shown and might be facilitated by immunosuppression aimed at limiting rejection, although this route may result in a detectable cancer in under 0.05% of graft recipients – usually melanoma.

Hammering the hallmarks

The hallmark features referred to previously not only distinguish normal from cancerous cells but thereby also represent attractive drug targets for treating or even curing cancers. In the modern era we now have a range of targeted anticancer drugs that specifically antagonize important molecular targets, such as growth factor signaling (BCR–ABL, EGFR, HER2 to name a few). In fact, an entire new vocabulary has been established to describe the application of these treatments and the changes in the cancer cell that accompany them. We will describe a select few here.

Cancer – Achilles' heel and Paris' arrow

The last decade has witnessed the beginnings of what is predicted to become a sea-change in cancer chemotherapeutics and arguably the single biggest paradigm shift since metaphor and hyperbole were first successfully mangled and combined in the cancer literature. What has driven so many of us to wax lyrical about a new dawn, about "Achilles'; heels," "oncogene addiction," and "personalized medicine"?

In a nutshell – we are excited by the identification in cancers of key signaling proteins essential for the maintenance of the cancer and the availability of drugs and ever more drugs that can relatively selectively inactivate those proteins. This is the realization in cancer therapy of the "magic bullet" model first proposed by Paul Ehrlich in the nineteenth century. Because this may arguably represent one of the biggest changes in thinking about drug design since the use of multidrug regimes first became mainstream in the 1950s and 1960s, we will devote the next few sections to this subject. To be fair, there have been examples of targeted therapies based on molecular grounds in the past, but they have never come so thick and fast and so specifically fuelled by detailed knowledge of the cancer-causing mutations and hypothesis-driven drug development. Thus, use of hormone manipulation such as anti-estrogens in breast cancer and antiandrogens in prostate cancer, somatostatin treatment for neuroendocrine tumors, and the use of HCG as a marker for treatment monitoring choriocarcinoma, all paved the way for today's targeted therapies and diagnostics.

Much current cancer research is directed towards finding and studying those specific molecular targets that are essential to the continued growth and survival of the cancer because these are obvious points of vulnerability that can be exploited in drug development. One, perhaps unexpected finding in cancer models that has excited great interest is that cancer cells often become highly dependent on some mutated growth signaling pathways. In other words, the cancer cells are said to manifest "oncogene addiction." Thus, constitutively active signaling through EGFR, for instance, suppresses other growth signaling pathways (oncogene amnesia) by various feedback mechanisms, leaving the cancer cell critically reliant on this one particular growth factor pathway (oncogene addiction). Importantly, normal cells either do not have these aberrant pathways or if they do they have other options and are relatively unaffected by their removal or inhibition. This explains why a targeted agent can have such initially powerful effects and comparatively little toxicity.

Incidentally, as oncologists are all obviously well versed in the classics, the weak spots of a cancer are often referred to as its "Achilles'; heel," and presumably by extension targeted therapies aiming to skewer that particular vulnerability should then be referred to as "Paris'; arrow" – well we'll see if the name Styx!

At present, the two main classes of new therapies which exploit such molecular knowledge are the humanized monoclonal antibodies and the tyrosine kinase inhibitors (TKIs). Although it took some time to convert hypothesis into reality, the successful treatment of the hitherto resistant chronic myeloid leukemia associated with the *BCR–ABL* oncogene with the TKI imatinib confirmed that cancers could respond to the specific antagonism of a single aberrant protein. Moreover, this acted as a proof-of-concept for the translation of progress in cancer molecular biology into new treatments and biomarkers. However, lest in mourning the plumage we forget the dying bird, it may prove salutary to remind ourselves that for most cancer sufferers, systemic treatments will still largely consist of DNA-damaging chemotherapy and – despite the often substantial associated side effects – usually to good effect.

Getting the GIST of oncogene addiction

Gastrointestinal stromal tumor (GIST) is a rare cancer but one that highlights many of the issues surrounding targeted therapies in cancers in general. Along with chronic myeloid leukemia, it was one of the original cancers treated with the then novel TKI imatinib mesylate (Gleevec). For many years, GIST was notorious for its lack of response to conventional chemoradiotherapy, yet much was known of the causative mutations, with most GIST-bearing mutations in *KIT* or, occasionally, *PDGFRA* or *BRAF* genes. Appreciating the pivotal role of KIT and the availability of imatinib, an inhibitor of KIT kinase, clinical trials soon followed and achieved a quite remarkable response in about 80% of patients with metastatic GIST. Along with parallel studies targeting the BCR–ABL kinase in chronic myeloid leukemia, these were the first examples of targeted therapy determined by genotype and have been followed by herceptin for breast cancers with HER2 mutations and others discussed later.

Cooking with ERBBs

An exemplar of the identification of key signaling pathways essential for cancer growth and survival is that regulated by members of the wider EGF receptor family. The ERBB family of proto-oncogenes comprise four closely related receptor tyrosine kinases, which include the epidermal growth factor receptor (EGFR), ERBB2 (also known as HER2), and ERBB3. These are powerful mediators of growth and survival signals in normal cells and in many human cancer cells. A further member, ERBB4, may actually be an inhibitor of growth. They become activated by ligand binding, which leads to dimerization of these receptors in homo- or heterodimers. EGFR is itself overexpressed in many non-small-cell lung cancers (NSCLCs) and this knowledge has been exploited in the increasing use of TKIs, such as erlotinib and gefitinib, in their treatment. In fact, finding the presence of EGFR mutations in some NSCLCs may identify those patients in whom TKI may be more effective than platinum-based chemotherapy.

ERBB2 is unusual in being constitutively in the active formation ready to bind to other ERBBs that have bound a ligand, and is aberrantly overexpressed in the evolution of many breast as well as subsets of gastric and ovarian cancers and may become aberrantly activated in some NSCLCs alongside EGFR. In fact, when present at high levels it can form spontaneously active homodimers and heterodimers resulting in ligand-independent replication- and survival-promoting signals which together

potently drive growth of the tumor. ERBB2 is amongst the best characterized and studied specific targets in cancer drug development, and much will be learned about how such knowledge has been exploited in developing new drugs for treating breast and other cancers. Thus ERBB2 signaling can be targeted by antibodies that prevent ligand binding (and may trigger immunity), TKIs, inhibitors of downstream signaling pathways, and cytotoxic antibodies.

Recently, it has been suggested that ERBB3 may be required for activation of the PI3K–AKT survival pathway and, moreover, may become overexpressed in some cancers or during therapy against other ERBBs, for example by amplification of MET, thus bypassing the need for other ERBBs. It is now the target of new drug development. Thus, knowledge of the presence of oncogenes such as NEU/HER, EGFR mutations/copy number, estrogen receptors, and others are already being used to guide treatments for individual cancer patients. Below we discuss some of the pioneering studies in targeted drugs.

Painting a portrait of cancer

Recent landmark studies have indicated that molecular analyses and gene expression profiling can identify key disease- and treatment-relevant molecules and even more complex relatively unique tissue "molecular signatures" that can be employed to improve our ability to predict disease prognosis and response to therapy. In fact, increasingly a combination of imaging techniques and molecular assays are being used to paint a portrait of cancer that brings out its true nature and reveals its particular obsessions and vulnerabilities.

Savile Row tailoring of cancer therapies

One aspect of recent progress in the area of biomarkers is the increasing realization that defining expression for single molecules may not be enough to accurately determine the optimal treatments and schedule for many patients, and great progress is being made in the simultaneous analysis of expression of multiple genes/proteins or in looking at genetic variation.

Earlier studies have employed a variety of high-throughput tools such as gene arrays, proteomics, and others to analyze changes and differences in expression of hundreds or thousands of genes/proteins between normal and cancer cells.

Thus, a gene expression signature was identified by global gene expression analyses in breast cancer that conferred a high risk of early development of metastases. Importantly, this "signature" was able to identify those individuals likely to progress among those otherwise generally regarded as low risk. This "poor prognosis signature," was shown to include genes regulating cell cycle, invasion, metastasis, and angiogenesis. Such studies provide support for the current "holy grail" of postgenome era medicine – namely disease fingerprinting and individualized medicine. There are still a number of barriers to overcome, and this is exemplified by the creation of whole new fields of scientific endeavor, badged under the heading of "systems biology" (see Chapter 20), which seek to develop the new techniques needed to derive more accurate and detailed information alongside new analytical techniques needed to handle large volumes of data.

One current hurdle to overcome relates to the loss of anatomical and topological information that accompanies many of today's high-throughput techniques. The differences between the output from destructive techniques that "mash up" a tissue in order to describe the molecular contents and that from microscopy techniques enabling co-localization of protein expression in the context of the intact topography and anatomy are akin to the differences between hearing a painting described on the radio and seeing it on television. Thus, many studies generally examine gene expression in "lumps" of tumor, which contain multiple cancer cells, stromal cells, and others and end up with a list of contents. This does not lessen the clinical utility of such whole-tumor studies, but imagine the "power" of a similar study looking at gene or, indeed, protein coexpression in individual cells on the canvas – with clear visibility not just of different cancer cells, but of stromal cells and vascular endothelial cells, and without disruption of the tissue anatomy.

Some preliminary steps have been made towards this ultimate goal but in general they have still ended up destroying the portrait to see what it is made of. Today, researchers are doing it one very small piece at a time. Thus, small bits of tumor can be isolated from tissue sections by means of laser capture microdissection. One study on breast cancer using this technique allowed identification of expression signatures that were remarkably similar across seemingly different clinical stages of cancer progression. This has fuelled notions that gene expression alterations conferring the potential for invasive growth might already be present in early preinvasive stages. In contrast to tumor stages, different tumor grades were found to be associated with distinct gene expression signatures, particularly between preinvasive and invasive.

Despite this progress, in most cases we are still unable to fully explain cancer behavior by such studies, and prognostic and treatment decisions are still often empirical. Even basic questions regarding cancer cell behavior and interactions with the microenvironment are unanswered. In particular, it is still far from clear just how clonal metastatic tumors actually are, and how individual metastases in the same patient are "related" to one other. Furthermore, the location in which evolution of the various mutations detected in metastases has occurred is still debated – in the primary or after spread. This likely varies from one cancer to another. Thus, in a study of over 200 human hepatocellular carcinomas and 7 metastatic liver lesions a MET-regulated gene expression signature (MET is associated with invasive behavior) was found in a subset of primary tumors and in all liver metastases, suggesting that the metastatic cells in this case originated from a clone within the primary and at least this metastasis-supporting mutation occurred before the cells left the primary tumor. The MET signature also correlated with increased vascular invasion and decreased mean survival time of hepatocellular carcinoma patients. Such poor prognosis signatures have also recently been reported for colon cancer, endometrial cancer, and NSCLC.

It must be remembered, however, that even in such studies "groups" of cells rather than single cells have been profiled; it is by no means certain that all the genes expressed apply to any individual cell. Tumors are usually genetically heterogeneous, and therefore tumor profiling, unless supplemented by single-cell analyses, may lead to erroneous conclusions, particularly if the assumption is made that all abnormalities detected apply to all individual tumor cells. Clonal expansion does not equate to all cancer cells being identical, simply that all cells will in some way carry the initiating genetic lesions alongside those additional mutations acquired during "cancer evolution." Moreover, with the increasing acceptance of the stem cell theory of cancer (discussed earlier), which implies that a small side

population of cancer-initiating cells carry the replicative and invasive potential, this is increasingly pertinent.

With this in mind, recent studies suggesting that single cancer cells from primary tumors may indeed carry the "poor prognosis" signature for metastases are very exciting, but will need confirming. Two recent papers using DNA sequencing to look at pancreatic cancers provide further food for thought. Both looked at clonal relationships between primary tumors and metastases in a number of different patients. In these studies, initiating mutations or rearrangements were identified in the primary tumors, including some that might drive amplification of cancer genes, such as telomere dysfunction and checkpoint disturbances. One study demonstrated that genomic instability frequently persisted after spread, driving parallel and even convergent evolution within cancer cells in different metastases. This also suggested that metastasis-initiating cells were genetically heterogeneous, supporting the contention that seeding metastasis requires mutations different to those supporting growth in the primary tumors. Furthermore, they also found that phylogenetic trees across metastases showed branches specific for a given secondary location. In the second paper it was also shown that clonal populations that seed the distant metastases were represented within the primary carcinoma, and had evolved from the original parental, nonmetastatic clone. Much of the genetic heterogeneity of metastases is simply a mirror for that already present in the primary carcinoma.

The two papers largely differ in terms of the degree of heterogeneity resulting within the primary or after spread. Mathematical analysis suggested that a decade or more might be required between the occurrence of the initiating mutation and the birth of the parental, nonmetastatic founder cell and a further 5 years for the acquisition of metastatic ability.

The pitfalls of tumor profiling

Increasingly, it is apparent that a good understanding of major genetic and epigenetic factors will still provide only a partial picture of disease. In practical terms, cancer patients with ostensibly identical clinical stages of disease (and probably even those with apparently similar genetic factors) may have markedly different treatment responses and overall outcome. In the same way that genomics offers the possibility of a more complete understanding of disease by describing multiple polymorphisms, so advances in molecular biology raise the possibility of going a step further. Cell behavior and disease pathogenesis ultimately arise through the differential expression of multiple genes and in turn by their protein products, in the diseased cells and also in other cells, neighboring and more distant, within the affected organism. At best, genomic sequences will have only a partial relationship to gene/protein expression, particularly as they will largely overlook epigenetic factors and moreover large-scale identification of polymorphisms may be far more difficult to comprehend than a molecular profile from a given cell/tissue.

A cancer protein expression profile

> You have made your way from worm to man, and much within you is still worm.
>
> *Friedrich Nietzsche*

Ultimately, it is proteins that determine phenotype. Not all genes are expressed in any given cell, and even of those genes expressed (for which mRNA is formed), alternative regulatory events may still take place after transcription that determine protein levels. Although considerable correlation exists between gene and protein expression, there are far more proteins than genes (Fig. 1.12). Thus, alternative splicing of RNA, posttranslational modifications, and enzyme activities can all contribute to the generation of a multitude of different proteins. Importantly, not all of these different proteins can therefore be directly inferred from examination of either the genome or even the transcriptome of a given cell at any given time. This has been the major impetus behind efforts to describe the cell proteome using mass spectrometry and other techniques (see Chapter 20).

There are now several contenders in the race to provide a tool that can enable the examination of molecular phenotype at high resolution and for multiple proteins simultaneously in their normal cellular or anatomical context. These include microscopy-based techniques such as the toponome imaging system (described in Chapter 20), in which thin-tissue sections are examined the co-localization of 30–100 proteins at a cellular and subcellular level, and variations of mass spectrometry imaging, which have lower resolution but do not necessarily require specific reagents to identify each protein (discovery techniques). Such techniques may bring us close to being able to finally look at the genuine portrait of cancer or at least a high-quality broadcast version! In Chapter 20 (systems biology) we discuss some of the exciting new techniques being used to look at single cancer cells within tumors and how systems biology will contribute to one day making individualized medicine and tailored therapy a reality.

The drugs don't work

Unfortunately, an addictive personality characterizes cancer cells and if they cannot get their normal EGFR fix then they either get it from somewhere else (a different drug-resistant mutation in EGFR occurs) or they get their growth hit from activating mutations in some other pathway, such as MET! In other words, oncogene addiction and acquired resistance to targeted treatment appear inextricably linked.

Lest we forget, however, it is worth noting that resistance to chemotherapy is not a new finding. The early chemotherapy pioneers, using generally cytotoxic drugs (at the opposite end of the targeted spectrum to imatinib), struggling to cure acute lymphoblastic leukemia in young children, soon realized that combinations of four drugs were needed to achieve remissions and that these needed to be repeated to achieve cures. Why? Because leukemia cells stopped responding to individual drugs or even small numbers of drugs, presumably by acquiring resistance. Even small numbers of surviving cells would then sooner or later repopulate. Two concepts were introduced – first, drugs kill proportions of cells and therefore many rounds of treatments may be needed to eliminate essentially all cancer cells, and second, cancer cells develop resistance. Resistance may relate to a variety of factors, including cancer cells finding sanctuary in areas where the drugs do not reach or work, acquiring mutations that enable them to avoid, exclude, or destroy the drugs, getting tougher and failing to die or even finding ways to avoid the activity of cancer-unfriendly immune cells. The concepts of specific pathway activation conferring resistance is not therefore really a new one, it is simply a byproduct of the specific nature of the new drugs,

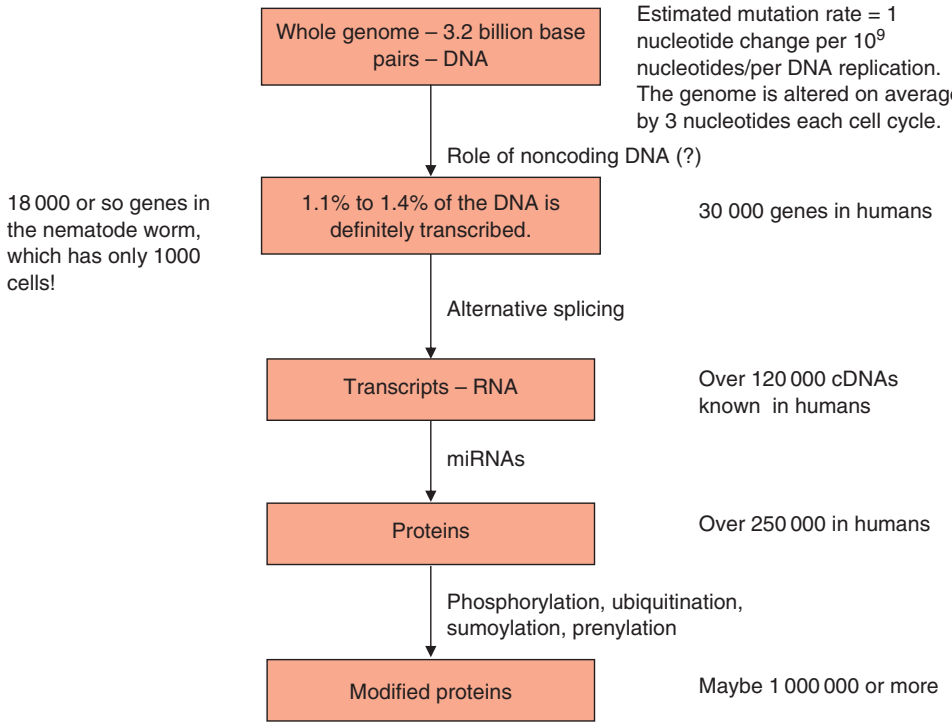


Figure 1.12 The complexity of cellular information flow in cancer. Although the issue of information flow seems hopelessly complex, there is much reason for hope. First, the availability of the reference genome for humans and many experimental models, alongside new technologies for analyzing the expression of multiple genes and proteins and appropriate techniques for analyzing and distributing experimental data will, it is hoped, result in major progress in "discovery science." Second, as many key genes/proteins have homologs in more primitive and experimentally amenable organisms we should have a much greater scope for functional studies.

which allow a simpler and more obvious route of escape for evolving cancer cells.

The addictive personality of cancer – synthetic lethality and non-oncogene addiction

To stretch the analogy further, an addictive personality may also result in cancer cells being addicted to more than one protein, many of which may not be oncogenes or even mutated at all. A good example of this is that breast cancer cells with defective homologous recombination (a form of DNA damage repair) are very sensitive to inhibitors of the enzyme poly(ADP-ribose) polymerase (PARP), whereas normal cells are not. This specific vulnerability to a drug inhibiting a specific target is referred to as "synthetic lethality" (a term shamelessly purloined from yeast genetics). In its original usage, synthetic lethality referred to the ability of a combination of mutations in two or more genes to kill a cell when a mutation in any one alone did not. In yeast cells a scientist would start with a cell carrying a nonlethal mutation and then test additional mutations one by one to find killing combinations. In some cases, such synthetic lethal interactions would identify how a cell may protect itself from the effects of the original mutation. This same technique has now been adopted to find new drug targets in cancer cells by using rapid gene knockdown screens using siRNA libraries. Synthetic lethality has been used in cancer to describe the killing of a cancer cell by a drug targeting the oncogene to which the cancer cell is addicted, but more usually refers to the targeting of a second protein to which the cancer cell is rendered dependent by another recognized mutation. Thus, the synthetic lethal interaction between defective BRCA and PARP has been exploited in breast cancer by use of PARP inhibitors; only breast cancer cells with defective BRCA are killed by these drugs.

On a related note, the ability of traditional chemotherapies to kill cancer cells more readily than normal cells has been referred to as genotype-dependent lethality; the totality of the cancer cell genotype/phenotype makes the cell vulnerable to DNA damage or cell-cycle paralysis.

Mechanism of origin rather than cell of origin – towards a new functional taxonomy of cancer

As we have discussed, cancers are traditionally classified on the basis of tissue of origin and this can be further refined to include cell of origin. However, as you will appreciate by now we are increasingly able to describe cancers according to the molecular alterations responsible for their development and required for their continued survival, and it will not have escaped your attention that these may sometimes be shared by cancers in different tissues. In fact, we are moving inexorably towards a new taxonomy of cancer in which diseases may be grouped not by tissue of origin but rather by common underlying disease mechanisms. The obvious exemplar would be breast and ovarian cancer, particularly those related to genomic, and therefore inherited, mutations in BRCA genes or, more recently, *RAD51D*. In these cases the mutations may illuminate the means by which tumorigenesis has proceeded, namely through an apical defect in the DNA

damage response (once a cell has lost the remaining functional allele). Moreover, this insight may also point to specific treatment target – a form of personalized medicine. Finally, the identification of the causative inherited mutation will enable the offer of genetic testing to relatives of affected cases, which may be used to predict family members at future risk of both types of cancer.

Arguably, this is a far more useful clinical definition than tissue of origin. In keeping with this new way of classifying cancers, ovarian and breast cancer related to inherited BRCA mutations will share more common features than will, for example, a triple negative and a *HER2*-related breast cancer.

Is it worth it?

> Now I saw, though too late, the folly of beginning a work before we count the cost, and before we judge rightly of our own strength to go through with it.
>
> *Daniel Defoe, Robinson Crusoe*

No discussion of diagnosis and treatment can take place without consideration of the overarching importance of economic considerations. There is no doubt about the challenge facing healthcare systems; around 12 million new cases of cancer were diagnosed in 2008 and cancers accounted for nearly 15% of all deaths globally. How are we meeting this challenge? First, by spending money on research; large pharmaceutical companies alone spend around US$100 billion per year, which is incidentally roughly the same as the annual cost to healthcare providers across Europe for treating cancer.

How do we quantify the cost to cancer patients? Measures have been devised which include both mortality and disability suffered by survivors. One composite used by WHO is the DALY (disability adjusted life years lost), which effectively equates to the loss of a healthy year of life. Another similar measure is that of quality-adjusted life years (QALY). These are particularly important in the United Kingdom, which, unlike the United States, widely uses health economics to ration available treatments in order to keep spending within often narrow budgetary constraints (save money). Thus DALYs can be balanced against treatment costs in order to decide which therapies will be provided by the state. Obviously, there is a risk of establishing a two-tier system, as the well-heeled can simply pay privately for the drugs not thought sufficiently good value for money by the state. It is not hard to imagine that the patient may put a rather higher value on their life and health than the state!

Thus, available treatments for cancer patients in many countries are not dictated simply by the speed with which academics and pharmaceutical companies can get new drugs delivered to cancer units, but much more by the willingness of healthcare commissioners and providers to pay for them (and let us not pass the buck completely to politicians, but also our own willingness – or that of our insurers – to pay for them directly or through increased taxation). Inevitably, economics raises the big question – how much is a life worth? And, lest we naively assume that everybody, even in the United Kingdom, gets the same level of healthcare, related questions such as how much is somebody else's life worth, how much is my life or that of my family worth? There are already large differences in views on this across different countries. For instance, cancer drugs account for around 10–20% of the direct costs of cancer care (about 5% of the total drug budget for all diseases). At present the United Kingdom spends effectively less on cancer drugs than any other large European economy, and this trend is increasing as uptake and spend on new drugs continues to be less than for France, Germany, Italy, or Spain. In fact, the United Kingdom spends 50% less per head of population than France and even Spain, in which cancer rates are lower, which reflects the relatively limited role of health economics in decision-making in these countries on the one hand and the preeminent role of this in the United Kingdom.

These questions are particularly pertinent to cancer, where costs have been spiraling out of control under the twin influences of increasing incidence and survival of cancer patients and high cost of treatments. Moreover, many feel that the marginal benefits of many of these costly treatments should encourage us to re-evaluate existing practice and closely scrutinize any new treatments. However, how easy is it to assess the value of a cancer drug, which may have shown a mean 6-month improvement in longevity in a group of cancer patients? Remember, trials are often conducted in high-risk groups who have failed on conventional treatments and often have late- or even endstage disease. Might these drugs not do better in the real world if used earlier and isn't any improvement in life expectancy or quality of life worth having? Clearly, as health resources are limited, somebody has to make difficult decisions or put another way implement rationing. What factors do you include in these decisions? Simple metrics – cost of treatments versus life years gained, societal benefits from returning somebody to work? Should the affluent avoid these compromises by simply paying for the drugs etc. themselves? Is this equitable? Do you treat those whose failure to comply with preventative advice on obesity, smoking, etc. has contributed to their eventual illness differently to those who become ill despite a healthy lifestyle? This happens already: active smokers and alcoholics are very unlikely, respectively, to get a coronary artery bypass graft or a liver transplant should they need it. How do you compare the value of renal dialysis in adults, stroke care for the elderly, and chemotherapy for children? Who is involved in these decisions?

Broadly, government and their representative organizations with varying degrees of political autonomy (such as NICE in the United Kingdom), professional societies, such as the American Society of Clinical Oncology or Cancer Research UK, licensing authorities such as the Food and Drug Administration, insurance companies, and, most of all, cancer specialists must fight for their corner as do representatives of all other medical and surgical specialities with varying degrees of success. Practitioners should not have to make individual rationing decisions day to day in their practices, as this will compromise the doctor–patient relationship, but should instead lobby and discuss to influence policy overall. But all too often this is unavoidable.

Conclusions and future directions

Early diagnosis is essential for most effective treatment and it is likely that advances in this area will produce the most extensive and immediate benefits for cancer patients. At least as important is reversing the more self-destructive lifestyle choices such as smoking and obesity, which account for a substantial number of cancers. In some cases where lifestyle change is undesirable or unlikely we may be able to prevent some cancers by vaccination or drug treatments.

Greater biological understanding of tumorigenesis is also important. Cancers arise by the stepwise accumulation of mutations and epigenetic factors that alter gene expression to confer the so-called "hallmark features" of cancer. The presence of inherited cancer-causing mutations will give a would-be cancer cell a headstart, but somatic mutations and epigenetic alterations are still needed for cancer development (Fig. 1.13). Variation in multiple genes when coupled with poor lifestyle choices (your own or those of others) increase risk of developing some cancers. It is likely, given the increasing susceptibility of progressing cancer cells to mutations, that not all such mutations are actually cancer-relevant. It is anticipated that improved knowledge about these various processes regulating aberrant gene expression and gene–environment interactions will lead to new preventive strategies and treatments aimed at specifically targeting the expression of genes/proteins "mission critical" for the initiation and progression of cancer.

The identification of key proteins to which the cancer cell has become addicted is already being translated into new therapies, as is the way in which resistance to these evolves during treatment. Increasingly, focus will likely shift towards an assault on a limited subset of specific cancer-promoting signaling pathways involved in survival, self-renewal/replication, and spreading and directing these at the ring-leaders within the tumor. In fact, it is hoped that a cancer could be arrested or even eliminated by assassinating a subpopulation of particularly malign cancer stem cells and/or nontumor cell collaborators within the stroma.

Of course, a note of caution is always recommended.

> "I am afraid," replied Elinor, "that the pleasantness of an employment does not always evince its propriety."
>
> *Jane Austen*

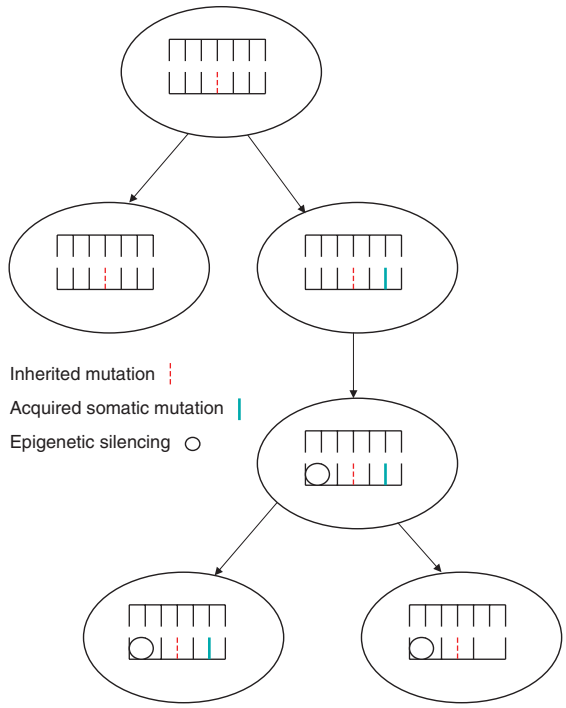


Figure 1.13 Tumorigenesis ultimately results from disordered gene expression. Tumor cells arise through aberrant expression of genes and the proteins they encode. This may result from mutations in the coding or noncoding regulatory regions of genes, which can be either inherited or acquired in somatic cells or even by major rearrangements of the chromosomes; epigenetic factors such as altered patterns of methylation and acetylation, which control the "accessibility" of genes for transcription. These events may in turn affect the stability and processing of RNA or proteins.

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Appendix 1.1 History of cancer

(see also: http://press2.nci.nih.gov/sciencebehind/cioc)

The difficulty in identifying traces of cancer in ancient remains and fossils inevitably makes a chronological survey of cancer difficult, and in particular largely precludes a reliable estimate of the prevalence of cancer until relatively recent times. Cancer has clearly existed for a very long time and skeletal metastases have been identified in archaeological specimens and a rectal cancer was found recently in an Egyptian mummy. At least one convincing report of a metastatic cancer has been reported in a dinosaur fossil, suggesting that cancer may have existed as long as complex organisms, but such findings are rare. There are few, if any, convincing fossil remains suggestive of cancer in Neanderthals or early humans.

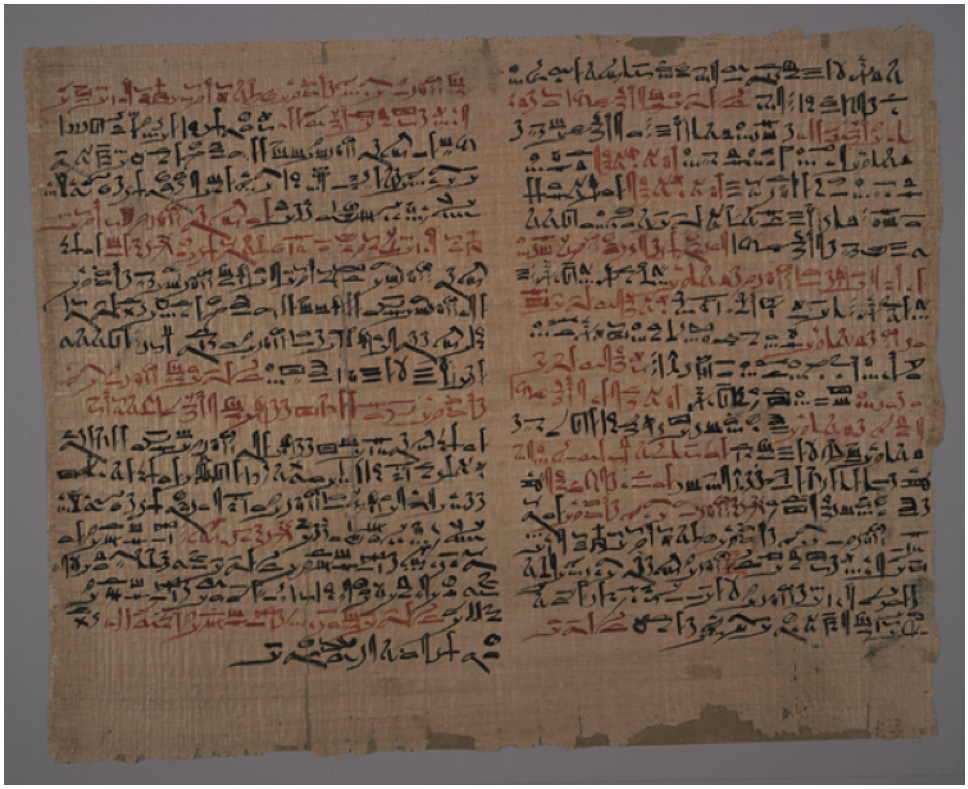
The key question is whether this scarcity of cancer-containing specimens is a result of the technical challenges in diagnoses and therefore the vagaries of paleopathology or, on the other hand, represents confirmation of the central importance on cancer pathogenesis of a modern lifestyle replete with environmental carcinogens, aversion to physical activity, and, ironically, an extended lifespan. The answer is not clear.

The widespread mummification of bodies in Ancient Egypt alongside the availability of written records offers greater opportunities to consider cancer in antiquity. Early Egyptian papyri from around 1600 BC, such as the "Edwin Smith" and "George Ebers" papyri, include descriptions of benign and malignant tumors and treatments based on castor oil and various animal parts, including pigs'; ears. Not that the Edwin Smith papyrus was particularly encouraging, as illustrated by this extract from case 45:

> If you examine a man having tumours on his breast . . . if you put your hand upon these tumours and you find them very cool, there being no fever at all therein . . . they have no granulation, they form no fluid, they do not generate secretions of fluid, and they are bulging to thy hand. *There is no treatment.* If you find tumours in any member of a man, you shall treat him according to these directions.

Nevertheless, many of the early written descriptions of cancer originate from the Classical Greek and Roman physicians Hippocrates and Galen, who laid the foundations for modern medicine by emphasizing that diseases were natural physical processes. In fact, we owe our names for cancer to Hippocrates, who first applied the terms *karkinos* and *karkinoma* (Ancient Greek for "crab") to various diseases, including cancers of the breast, uterus, stomach, and skin. Cancer is the Latin equivalent. Interestingly, although Galen performed some early surgical interventions for cancer, he maintained that cancer was generally best left untreated, a view that appears to still find favor with some health economists. However, Galen also believed that diseases resulted from imbalances in the four bodily "humors" (blood, phlegm, yellow bile, and black bile), which were also responsible for differing temperaments such as melancholy!

Humoral theory, first raised by Hippocrates around 2500 years ago, and extended by Galen, remained the central tenet of essentially all Western medicine until the 1800s. Given the prevalence of this view for around 2000 years, it bears a brief diversion to



Section of the Edwin Smith papyrus. From the National Library of Medicine http://archive.nlm.nih.gov/proj/ttp/smith_home.html.

discuss it. The human body was believed to comprise a mix of the four humors: black bile (or melancholy), yellow or red bile, blood, and phlegm. The balance of these varied from individual to individual and as long as they were in the correct balance for you, you remained healthy (the first example of individualized medicine perhaps). The humors were directly linked to temperaments: melancholic, sanguine, choleric, and phlegmatic – thus also encompassing the links between mind and body.

Unfortunately, relatively little progress was recorded during the so-called Middle Ages (from the fall of the Roman Empire until the Renaissance). Although clearly in the Arab world, Moorish Spain, Constantinople, and in the West in monastic communities, much classical learning was preserved and recorded for the future benefit of Renaissance scholars. This generally negative view of human progress in the Middle Ages as being largely the copying and preservation of classical texts for the future benefit of Renaissance scholars is rather overstated, as illustrated by an intriguing quotation from Theodoric, Bishop of Cervia (1267) – "The older a cancer is, the worse it is. And the more it is involved with muscles, veins and nutrifying arteries, the worse it is, and the more difficult to treat. For in such places incisions, cauteries and sharp medications are to be feared."

Much important scholarship was also taking place in the Arab world, not least of which was laying the foundations for modern mathematics. With respects to cancer, the insightful writings of two prominent Arab scholars have been recorded. Thus, to quote Avicenna (981–1037):

> The difference between cancerous swelling and induration. The latter is a slumbering silent mass, which . . . is painless, and stationary. . . . A cancerous swelling progressively increases in size, is destructive, and spreads roots which insinuate themselves amongst the tissue-elements;

and Albucasis (1050):

> The Ancients said that when a cancer is in a site where total eradication is possible, such as a cancer of the breasts or of the thigh, and in similar parts where complete removal is possible, and especially when in the early stage and small, then surgery was to be tried. But when it is of long standing and large you should leave it alone. For I myself have never been able to cure any such, nor have I seen anyone else succeed before me.

From classical times until the late Renaissance, when Vesalius and artists such as Michelangelo and Leonardo da Vinci developed an interest in anatomy, cancer was still believed to be caused variously by Acts of God or still, in deference to Galen, by an excess of black bile. Although still believed to be incurable, a wide variety of arsenic-containing preparations were employed to treat it. Based on his observations in Austrian mines, Theophratus Bombastus von Hohenheim, better known as Paracelsus, described the "wasting disease of miners" in 1567. He proposed that the exposure to natural ores such as realgar (arsenic sulfide) and others might have been causing this condition. Paracelsus was actually among the first to consider a chemical compound as an occupational carcinogen. Paracelsus was probably the first prominent objector to Galen's humoural doctrine, and instead proposed that mineral salts when concentrated in a particular part of the body and unable to find an outlet, were the real cause of cancer.

The beginnings of recognizably modern science took place in the seventeenth century; William Harvey described the continuous circulation of the blood, finally resulting in the rejection of the humoral theory of disease, and cancer was no longer attributed to bile. A contemporary of Harvey, Gaspare Aselli identified the lymphatic system, which he suggested as a primary cause of cancer. However, on the basis of this discovery, René Descartes developed a new theory, termed the "sour lump" theory in 1652, whereby it was suggested that lymph became hard through some congealing process and formed a scirrhus. If this fermented (i.e. became acid or sour) then a cancer would develop. Surgery for cancer now began to include removal of the lymph nodes when enlarged and near the tumor site. A renowned German surgeon, Fabricius Hildanus, removed enlarged lymph nodes in breast cancer operations, but in the absence of either septic techniques or anesthetics it was an extremely hazardous procedure.

In the eighteenth century, oncology became a recognized discipline, with early experiments conducted. The French physician Claude Gendron (1663–1750) concluded after 8 years of research that cancer arises locally as a hard, growing mass, untreatable with drugs that must be removed with all its "filaments." The Dutch professor Hermann Boerhaave believed inflammation could result in a scirrhus, or tumor, capable of evolving into cancer. John Hunter, one of the earliest modern surgeons, taught that if a tumor were movable, it could be surgically removed, as could resulting cancers in proper reach. If enlarged glands were involved, he advised against surgery.

Two eighteenth-century French scientists, physician Jean Astruc and chemist Bernard Peyrilhe, conducted experiments to confirm or disprove hypotheses related to cancer. Their efforts may appear eccentric to us now, but they helped establish the discipline of experimental oncology. For example, in 1740 Astruc, a professor of medicine at Montpellier and Paris, sought to test the validity of the humoral theory by comparing the taste of boiled beef-steak with that of boiled breast tumor; he found no black bile–like taste in the tumor – he may also have had a lasting influence on French culinary practices! Peyrilhe attempted to demonstrate an infective cause for cancer by injecting human cancer tissue into a dog. The resultant infected abscess (no cancer!) resulted in a housemaid drowning the poor dog to end its misery.

Later in the same century, two English physicians – John Hill and Percival Pott – described the occurrence of cancerous alterations in the nasal mucosa and at the skin of the scrotum in a few patients, and linked it with local long-term exposure to snuff and repetitive local contamination by soot, respectively.

The nineteenth century heralded the beginnings of modern biology. Virchow focused pathology on the cell; and anesthesia and antisepsis improved surgery. Oncology progressed as Röntgen described X-rays, the Curies isolated radium, and Müller observed abnormalities of cancer cells. By the mid-nineteenth century, French and Italian researchers had found that women died from cancer much more frequently than men, and that the cancer death rate for both sexes was rising. Domenico Rigoni-Stern concluded that incidences of cancer increase with age.

Throughout the early decades of the twentieth century, researchers pursued different theories of the origin of cancer. Theodor Boveri, professor of zoology at Wurzberg, proposed that cancer was due to abnormal chromosomes. This was remarkably prescient given that it was more than 40 years before the discovery of the structure of DNA. A viral cause of cancer in chickens

was documented in 1911, and both chemical and physical carcinogens were conclusively identified. Radium and X-rays were employed against cancer early in the century, and it was found that X-rays selectively damaged cancer cells, causing less harm to other tissues. As safe levels of dosage were determined, the therapy became standard. Chemical- and radiation-induced cancers were first reliably confirmed as carcinogens. While the smoking–cancer link was noted in the 1930s, causality was only proven following extensive epidemiological studies in 1950.

Molecular biology has revolutionized both medicine and cancer research; following the identification of the structure of DNA by Francis Crick and James Watson in 1953, the genetic code was soon broken, and the foundations were laid for much of what is discussed in this book.

We conclude with two quotes, illustrating how far we have progressed in cancer therapy:

Cancer is an uneven swelling, rough, unseemly, darkish, painful, and sometimes without ulceration . . . *and if operated upon, it becomes worse* . . . and spreads by erosion; forming in most parts of the body, but more especially in the female uterus and breasts. It has the veins stretched on all sides as the animal the crab (cancer) has its feet, whence it derives its name.

Paul of Aegina (625–690)

A carcinoma does not give rise to the same danger [as a carbuncle] *unless it is irritated by imprudent treatment.* This disease occurs mostly in the upper parts of the body, in the region of the face, nose, ears, lips, and in the breasts of women, but it may also arise in an ulceration, or in the spleen. . . . At times the part becomes harder or softer than natural. . . . After excision, even when a scar has formed, none the less the disease has returned, and caused death.

Aulus (Aurelius) Cornelius Celsus (25BC–AD50)