MEDICAL RADIOLOGY

Radiation Oncology

L.W. Brady
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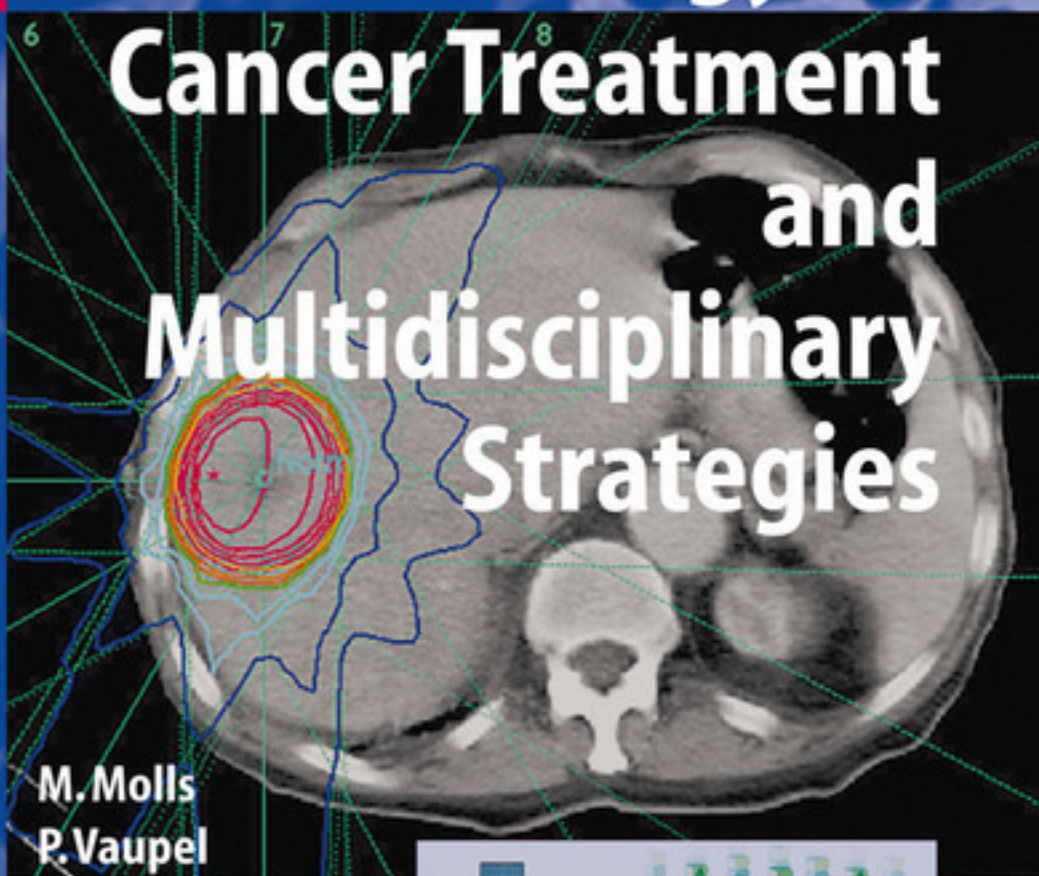
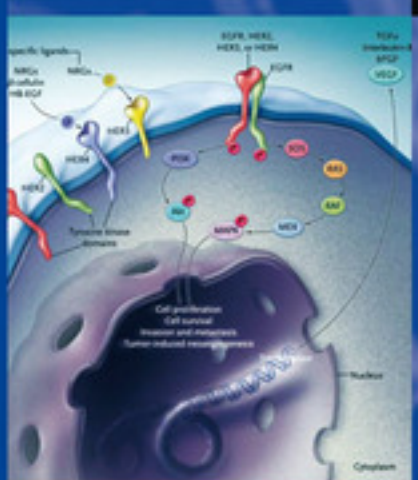
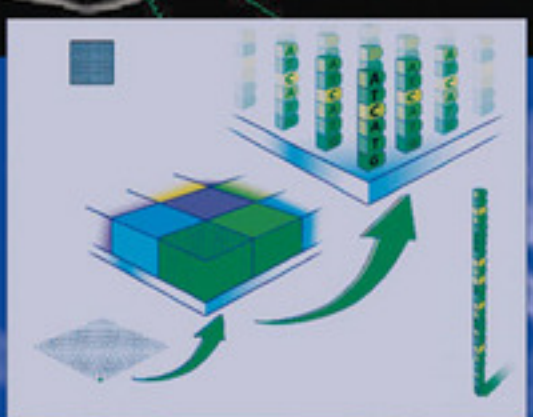
M. Molls · C. Nieder

The Impact of Tumor Biology on Cancer Treatment and Multidisciplinary Strategies







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The Impact of Tumor Biology on Cancer Treatment and Multidisciplinary Strategies

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Foreword

The rapidly changing concepts in radiation oncology with the development of more precise instrumentation for delivery of radiation therapy and a greater emphasis on hypofractionation technologies require a very intimate knowledge of tumor biology and the influence of various biologic factors on dose distribution within the tumor in terms of homogeneity as well as prevention of any late effects on normal tissue surrounding the tumor itself. Not only are these major factors in clinical practice but also the known factors of inhomogeneity of cancer cells, the impact of microenvironment in terms of radiation effect, and host factors make it mandatory to design therapeutic strategies to improve the outcome and to diminish any potential short-term or long-term risks from the radiation therapy.

The authors have developed an outstanding text that deals with these strategies and how they would impact on established and emerging new technologies and treatment. The context of the presentations within a multidisciplinary combined modality therapy program is incredibly important.

In this volume, various topics are reviewed including tumor genesis, cell proliferation, angiogenesis, the physiologic characteristics of malignant tissues, invasion and adhesion, the route and role pursued in the development of metastasis, and the role of the human immune system in cancer prevention and development.

Important chapters focus on cancer diagnosis and treatment along the basic principles of chemotherapy, radiotherapy, and molecularly targeted therapy. The presented rational adaptations allow for the design of translational studies and become increasingly more important as a better understanding is gained of gene expression profiling, gene transfer and silencing, proteomics and molecular imaging and their impact on the development of treatment programs.

The authors' aim is to educate and inspire those who devote most of their work to research in cancer and its clinical treatment. It represents an outstanding presentation in these regards.

Philadelphia — Luther W. Brady
Hamburg — Hans-Peter Heilmann
Munich — Michael Molls
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Preface

Numerous developments in molecular biology and information technology over the past decade have led to an explosive growth in cancer biology research. Much of the research has focused on the underlying mechanisms of carcinogenesis, tumor progression and metastasis. Knowledge gained from this research has led to the development of new classes of drugs that target specific pathways known to be involved in one or more of the processes that may be altered as part of the malignant phenotype.

Radiation oncology as a specialty has benefited from this technological revolution, and it is now possible to target therapies much more precisely and safely than in the past. It is critically important, however, that the radiation oncologist becomes knowledgeable not only about new developments in radiation biology, but also about cancer biology in general. In fact, radiation biology has embraced molecular biology to such a degree that there are now few classically trained radiobiologists remaining on the faculties of many radiation oncology departments.

The purpose of this book is to provide the practicing radiation oncologist, as well as those in training, with a concise overview of the most important and up-to-date information pertaining to tumor biology as it impacts on cancer treatment. This information is not limited to that directly related to the interaction of radiation with cells and tissues, for it is important that the radiation oncologist have a broader understanding of tumor biology.

It is the intent of the editors to provide chapters from experts in not only the basic sciences, but also in the translational application of key basic biological concepts. Thus, the book contains chapters on the fundamental basic principles of cancer biology, such as tumorigenesis, cell growth and proliferation, angiogenesis, tumor physiology, the biology of metastasis and the role of the immune system. More clinically related topics, such as molecular and biological imaging and molecular targeted therapies for both cancer treatment and normal tissue injury, are also included. In order to be able to read and understand the latest literature, it is important to have an understanding of the principles behind some of the latest tools employed by scientists to conduct their research. To that end, chapters describing techniques such as gene expression profiling, gene transfer and gene silencing are also included.

We hope that the reader will find this book a useful guide to the molecular era of cancer biology and to the implications of increasing biology knowledge of personalized cancer therapy, particularly as it applies to the field of radiation oncology.

Munich — Michael Molls
Mainz — Peter Vaupel
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Tumorigenesis

1

Michael J. Atkinson and Soile Tapio

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KEY POINTS

- Analysis of the DNA of tumor cells reveals that a finite number of gene mutations are responsible for the transmission of the phenotypic changes characteristic of the tumor. These mutations may have arisen sporadically through misrepair of endogenous DNA damage from oxidative stress and DNA replication errors, or through mistakes in somatic recombination events. Alternatively, they may be induced exogenously through the DNA-damaging action of environmental agents such as ionising radiation and UV light.
- Failure of the damage control processes to correct the damage before it is incorporated permanently into the genome during replication is critical.
- In addition to the intragenic mutations, there is a range of additional mechanisms whereby the genome may become perturbed during tumor development. Alterations in the copy number of cellular genes are common in human tumors. Both allelic gains and losses are encountered. Amplification of genetic regions may take the form of intrachromosomal duplications, leading to the in situ amplification of a gene with oncogenic properties at its normal chromosomal location. Transcription of the amplified gene complex subsequently leads to overexpression of the gene product. Alternatively, the amplification may occur extrachromosomally, leading to the formation of multiple copies of chromosomal fragments (double minutes).
- The spectrum of mutational events in tumor cells can also include chromosomal translocation and inversion events leading to the structural rearrangement of parts of the genome. This may result in a fusion of two unrelated gene

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fragments, creating a chimeric gene instructing production of a protein with abnormal function. Alternatively, the rearrangement may transpose an endogenously active promoter with coding sequences from a gene that is normally either tightly regulated or transcriptionally silent in the tissue. This form of mutation leads to the inappropriate expression of the protein.

- Two non-mutational events are also implicated in the changes in gene expression during oncogenesis. In the first situation, transcriptional silencing of an essential tumor suppressor gene is associated with non-mutational changes to the structure of the gene promoter region. Changes in the methylation status of individual nucleotides of the DNA as well as to the methylation and acetylation status of the DNA-binding histone core proteins are involved in regulating local gene expression. A second non-mutational event is gene silencing through endogenous RNA-binding microRNA molecules.
- Oncogenes are genes that, through the action of the proteins they encode, cause cancer when transcribed. Oncogenes arise through the mutation of normal cellular genes with regulatory activities called proto-oncogenes.
- Tumor suppressor genes encode proteins that are responsible for control processes essential to limiting cell proliferation. They act upon pathways involved in growth control, cell cycle regulation and the maintenance of cell integrity (DNA repair and apoptosis).
- Carcinogens include a number of different substances that are directly involved in the initiation or promotion of cancer in humans. The nature of carcinogens varies from radiation to chemical substances, bacteria and viruses.
- Evolving concepts of tumor stem cells, the regulation of coordinated expression programmes by non-translated microRNAs and the role of the tumor microenvironment are just three areas where new knowledge is opening up possibilities for the diagnosis and treatment of malignant disease.

Abstract

Tumor cells possess a range of inherited phenotypic features that distinguish them from normal cells. They acquire the ability to undergo almost continual unregulated growth, resist cytotoxic chemicals and are able to metastasise from their initial locations to proliferate in inappropriate tissue compartments. This chapter describes the early stages of tumorigenesis, starting with genetic mutations and alterations in gene expression and biological signalling, and finally discusses inherited or environmental factors accelerating the initiative process to malignancy.

1.1 Introduction

The scientific search for the cause of cancer can be traced back to Hippocrates. His suggestion that an imbalance in the bodily fluids was the cause of cancer predated both the cellular theory of Johannes Müller and Rudolf Virchow and the oncogenetics of Vogelstein and colleagues. The Hippocratic view remained the conventional wisdom for generations, but was rapidly discarded in favour of more evidence-based models (Fig. 1.1). Maybe, given the importance now ascribed to the local tissue microenvironment in cancer, we should give more credit to Hippocrates.

After cancer was recognized as a cell-based disease, scientific effort focussed on understanding the processes involved in the genesis and behaviour of the abnormal cells. Whilst the origins of the cellular building blocks of tumors can be traced back to an apparently normal parental tissue, cancer cells clearly evolve unique phenotypic characteristics. Insight into potential mechanisms behind this process came from the early epidemiological studies by Percival Pott, Bernardino Ramazzini and others, who demonstrated exogenous causes for some cancer through infection, wounding or noxious chemicals (McDermott et al. 2007; Aronson 2007; Breasted 1922). The seminal study of Theodor Boveri, suggesting that tumors arise through abnormal distribution of chromosomes, focussed attention upon the genome (Manchester 1995; Harris 2008). Although Peyton Rous almost simultaneously established that the malignant phenotype could be transferred to normal cells in tumor cell extracts (Vogt 1996), the discovery of the central role of genetic material in the process had to await the explosion of interest in molecular biology that followed the clarification of the structure of DNA. This new era saw the identification of tumor-inducing genes within the genome of oncogenic viruses, the discovery that these viral genes were in fact mutated derivatives of cellular genes and that endogenous mutation of these very same cellular genes could give rise to cancers.

Although it was comforting to assume that a simple gene mutation underlies the development of cancer,

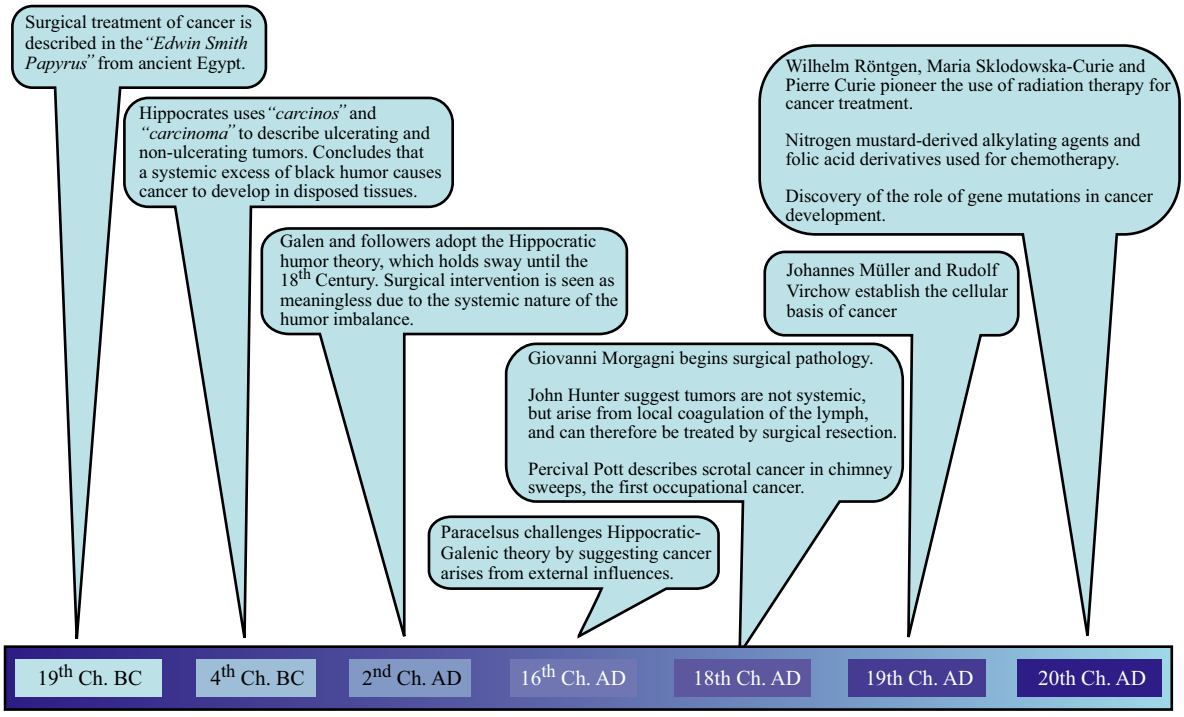

Fig. 1.1. Development of cancer biology over the centuries

more recent developments suggest that the reality is much more complex. Thus, the last decade has seen the realization that a host of other factors, such as epigenetic regulation, inherited susceptibility and changes in the local microenvironment, can all play a role in the development of a cancer. This expansion of our understanding of the carcinogenic process has many implications for the application and development of therapeutic strategies.

1.2 Early Mutational Events in Carcinogenesis

1.2.1 Alterations of the Genetic Code

Analysis of the DNA of tumor cells reveals that a finite number of gene mutations are responsible for the transmission of the phenotypic changes characteristic of the tumor from one cell to the other during cell division. These mutations may have arisen sporadically in a somatic cell through misrepair of endogenous DNA damage arising from oxidative stress and DNA replication errors, or through mistakes in somatic recombination events. Alternatively, they may be induced exogenously through the DNA-damaging action of environmental agents, such as ionising radiation, UV, and mutagenic alkylating or intercalating agents. Failure of the damage control processes to correct the damage before it is incorporated permanently into the genome during replication is critical.

Infrequently, the critical alteration in gene function may be transmitted to an individual from a parent through the germ line, in which case the mutation can result in a familial (heritable) cancer syndrome, such as retinoblastoma or one of the multiple endocrine neoplasias.

Mutations involving damage to only small regions of the genome that result in phenotypic change are usually intragenic and are limited to only a single gene. The smallest mutations involve a single base, either resulting in a nucleotide exchange or insertion/deletion of one base (frame-shift mutation). The consequences for the gene sequence of such mutations are determined by the context of the altered base. If it is present within a codon, the genome-encoded amino acid may be substituted, which may sometimes result in catastrophic changes to the protein sequence through substitution of an inappropriate amino acid into the protein chain. Some substitutions may have only a modest effect upon

phenotype or may even leave the encoded amino acid unchanged (silent mutations). Occasionally, the single base change may generate a premature stop codon, truncating the protein, which frequently leads to rapid degradation of the abnormal protein by the misfolded protein recognition system in the endoplasmic reticulum and the proteasome.

Insertions and deletions of a single base alter the reading frame of the gene. As most genes have evolved with multiple stop codons protecting the two non-coding frames, the frame-shifted sequence will most probably contain a stop codon close to the position of the insertion/deletion. In some infrequent instances, the mutated single base may lie in a critical structural element of the gene, such as the promoter site regulating gene activity, or in a recognition site critical for RNA processing, for example splice site mutations resulting in exon skipping deletions in the E-cadherin gene (Becker et al. 1993).

In addition to the intragenic mutations described above, there is a range of additional mechanisms whereby the genome may become perturbed during tumor development. Alterations in the copy number of cellular genes are commonly described in human tumors. Both allelic gains and losses are encountered, and their biological consequences are described elsewhere in this review. Amplification of genetic regions may take the form of intrachromosomal duplications, leading to the in situ amplification of a gene with oncogenic potential. Transcription of the amplified gene complex subsequently leads to overexpression of the gene product. Alternatively, the amplification may occur extrachromosomally, leading to the formation of multiple copies of chromosomal fragments (double minutes) containing one or more transcriptionally active genes with an oncogenic capacity.

The spectrum of mutational events in tumor cells can also include chromosomal translocation and inversion events leading to the structural rearrangement of parts of the genome. This may result in a fusion of two unrelated gene fragments, creating a chimeric gene instructing production of a protein with abnormal function. Alternatively, the rearrangement may transpose an endogenously active promoter to coding sequences from a gene that is normally either tightly regulated or transcriptionally silent in the tissue. This form of mutation leads to the inappropriate expression of the protein, for example, in parathyroid tissue where the CCND1 (cyclin D1) gene is placed under the control of the highly active parathyroid hormone gene promoter (Arnold et al. 2002). This is also seen in thyroid tissue where the transcriptionally inactive glial-derived neurotrophic factor receptor (RET) tyrosine kinase gene is placed under the control of one of a number of different promoters active in thyroid tissue (Santoro et al. 2004). As a result of this translocation event, the neuroendocrine tissue-restricted RET protein is produced in thyroid cells and delivers cell proliferation signals in a ligand-independent manner (see below).

Functional translocations are also frequent in the lymphoid and myeloid lineages, presumably due to the propensity of these cells to undergo chromosomal rearrangements during immunoglobulin and T cell receptor maturation. Failure to restrict the high level of chromosomal rearrangement activity to the correct locus may explain the abundance of such alterations in immature stages of the lineages. In solid tumors translocations are seen primarily in the endocrine tissues mentioned above and in the paediatric tumors rhabdomyosarcoma and Ewing's sarcoma, both of which involve activation of genes regulating developmental pathways. Translocations are reported less frequently in other solid tumors, and here their biological relevance remains uncertain. Significantly, in none of the solid tumor types showing translocations is there any evidence for endogenous chromosomal rearrangement processes that could explain the phenomena.

Two non-mutational events are also implicated in the changes in gene expression during oncogenesis. In the first situation, transcriptional silencing of an essential tumor suppressor gene is associated with non-mutational changes to the structure of the gene promoter region. Changes in the methylation status of individual nucleotides of the DNA, as well as to the methylation and acetylation status of the DNA-binding histone core proteins, are involved in regulating local gene expression. A second non-mutational event is discussed below, where gene silencing through endogenous RNA-binding microRNA molecules has been suggested to be an additional step in transcriptional control, leading to silencing in a post-transcriptional manner.

An altogether different mutational mechanism is seen almost exclusively in animal model systems, where insertion of retroviral sequences or retroviral-like elements into the genome results in the disruption of cellular genes. In humans, the role of insertional mutagenesis is less clear. Retroviral insertion leading to proto-oncogene overexpression has been implicated in the development of retroviral gene therapy-associated lymphoproliferative malignancies in a small number of cases. Nevertheless, the general applicability of this mutational mechanism for human cancer is unclear, and it is certainly uncommon. In addition to retroviral insertion, viruses have evolved a range of strategies for productive infection of mammalian cells that subvert defence and regulatory pathways. As a consequence of

these actions, the viral proteins elicit an oncogenic action through growth stimulation, suppression of apoptosis or inactivation of endogenous tumor suppressor gene function.

1.2.2 Events Accompanying Progression

Mathematical and molecular studies on tumor tissues have each established that tumors can arise and develop through a series of intermediate stages. The clonal expansion paradigm suggests that discrete stages arise through evolutionary selection of appropriate phenotypes that are themselves defined by mutational events. Histopathological studies deliver a partially convergent concept, where morphologically distinct stages of tumor formation and development are discernable in almost all tumor entities. The combination of the morphological models of tumor development and analysis of molecular events suggests that tumor development indeed follows a series of steps from pre-cancerous lesions (hyperplasia, atypical hyperplasia) that lead either directly or indirectly to full neoplasia (infiltrative and metastatic growth). During this progression, the normally differentiated phenotype may become either partially or completely lost (Walch et al. 2000).

Estimates of the number of mutations and steps that are required to create a full malignant phenotype vary wildly. In vitro studies suggest that mutation of as few as three key genes is sufficient, whilst massive DNA resequencing studies of tumor cell genomes have revealed hitherto undiscovered complexity in the magnitude and diversity of DNA alterations; however, it remains unclear which of these, if any, are required for the acquisition of a malignant phenotype (Sjoblom et al. 2006). Three conceptual models can help in partly reconciling these differences. Kinzler and Vogelstein suggested, at least for the model of colon carcinogenesis, that there is a linear evolution of the cells within the developing tumor, which follows a well-circumscribed and sequential series of events (Vogelstein et al. 1988; Vogelstein and Kinzler 2004). Each step in their model is represented by the mutation of a single key gene. However, the analysis of the gene alterations present in different areas of some tumors shows that some clones lack the full compliment of gene mutations. This may indicate that a simple linear monoclonal evolution is not always followed (Kuukasjarvi et al. 1997). An alternate view to the Vogelstein model is that mutations are acquired in a cumulative manner, with some clones in the tumor acquiring mutations that lead to them branching off to an evolutionary dead end and others only being required at specific points in the tumor development. Hanahan and Weinberg (2000) have suggested that key cellular pathways related to functional changes in tumor cell biology are individually targeted by mutational events, explaining how the development of malignancy can result from a finite number of mutations. Finally, systems theory and pathway analysis suggest that each functional activity of the cell described by Hanahan and Weinberg requires multiple hits to remove back-up and alternative pathways. It is, however, worthy of note that tumor cells cannot tolerate wholesale genomic alterations; consequently, there cannot be an unlimited number of mutations as some functional pathways are essential for continued cell survival.

A discrepancy of orders of magnitude between the sporadic rate of mutational activity observed in cells and the level of mutations found in tumors has prompted Loeb (2001) to suggest that a key process in tumor cell development must be the acquisition of a mutational activity (mutator phenotype, loss of caretaker function). Although tumor suppressor and apoptosis genes could be considered candidate mutator genes, no convincing evidence for a specific increase in mutation rate due to loss of these genes has been presented. Genes involved in maintaining genomic integrity, such as the DNA mismatch repair genes, whilst implicated in cancer susceptibility, provide no clear evidence of mutator-gene driven genome changes.

1.2.3 Proliferation Modifying Genes

A major category of the genes influencing cell proliferation contains members of signalling pathways involved in the regulation of cellular growth. At the cell surface this can be seen by the uncontrolled production of stimulatory growth factors, the abnormal expression of growth factor receptors or the production of a mutated form of the receptor that has acquired the capacity to autonomously engage and activate the downstream intracellular signalling cascade. A related functional set of tumor genes is that involved in the transmission of the growth-regulating signal to the transcriptional apparatus, which includes signal-transducing kinases and transcription factors.

An additional group of proliferation genes plays a role in steering the transit of cells into, through and out of the cell cycle. Inappropriate functioning of these genes leads to uncontrolled cell cycle activity and the failure of proliferating cells to differentiate. In the case of cell cycle checkpoint control genes, this can allow cells with non-repaired DNA damage or chromosomal

aberrations to continue through the cycle, yielding genetically aberrant daughter cells. Failure to eliminate damaged cells is an additional feature of the mutations influencing a further set of cancer genes, those involving the cellular pathways regulating programmed cell death (apoptosis and anoikis, a form of apoptosis that is induced in anchorage-dependent cells detaching from the surrounding cells and/or matrix). The failure of tumor cells to initiate a normal apoptotic death response after stress and/or mutation of DNA, or to initiate apoptosis after loss of cell–cell and cell–matrix contact, can involve inactivation of the intrinsic (mitochondrial) pathway and extrinsic (ligand-receptor) apoptosis-inducing pathways. This can be brought about by inappropriate overexpression of anti-apoptotic proteins or by inactivation of pro-apoptotic proteins. More recently, the protective sequestration of cells bearing oncogenic gene mutations into a pathway of oncogene-induced senescence (OIS) has been described. The regulation of this pathway is poorly understood, but escape from growth restrictions imposed by the activation of the senescence programme appears to be a critical step in oncogenesis and may involve overcoming cell cycle arrest by removing expression of the p16 cyclin-dependent kinase inhibitor. It remains to be seen which other protein activities regulate entry and exit from OIS and how mutations of these genes influence tumorigenesis.

1.2.4 Acquisition of the Invasive/Metastatic Phenotype

Although changes in proliferative regulation pathways are critically important, the acquisition of an invasive/metastatic phenotype is a major step in solid tumor formation. The necessary changes in gene expression may occur through mutation or through changes in more global programmes of cell regulation, such as the epithelial to mesenchymal phenotypic transition (EMT). Tumor invasion into surrounding tissues requires distinct phenotypic alterations. Loss of cell-specific adhesion allows tumor cells to detach from neighbouring cells and the underlying extracellular matrix. This may be accompanied by upregulation of an alternative programme of adhesion, allowing the tumor cell to adhere to anomalous cells or matrixes (e.g. a switch from epithelial-specific E-cadherin to the mesenschymal-cell specific cadherins in breast cancer) (Sarrio et al. 2008). At the same time as acquiring an abnormal adhesive profile, the tumor cells may also develop a programme allowing for the degradation of the surrounding matrix proteins. Here, overexpression of specific proteases may facilitate local destruction of matrix that allows the non-adherent tumor cell to exit the parental tissue and migrate (Wagner et al. 1995). Recent evidence suggests that the mobilisation of tumor cells may be driven by local gradients of cell- and tissue-specific chemokine molecules. Changes in the expression pattern of surface chemokine receptors of tumor cells may permit them to respond to a different chemokine milieu and has been suggested to be partly responsible for homing of tumor cells to specific distant sites such as bone marrow (Kulbe et al. 2004). Separation of the tumor cell from surrounding parental tissue would normally be expected to initiate the anoikis programme of apoptosis, but as described above, this pathway is inactivated as part of the loss of proliferative regulation. The final stage in malignant growth, the acquisition of the capacity to generate new blood vessels that infiltrate the tumor and oxygenate the expanding cell mass, angiogenesis, is discussed in other chapters of this book.

1.3 Inherited Susceptibility

Within a population there is a proportion of individuals who are predisposed to develop cancer, either as an apparently sporadic disease or in response to an environmental challenge, such as exposure to tobacco smoke or ionising radiation. The abnormally high frequency of some tumor types within related members of large families provided evidence that cancer is, in some circumstances, a heritable disease. Genetic linkage studies of these families has revealed that a number of these cancer syndromes occur as simple Mendelian traits, usually with a highly penetrant dominant pattern of inheritance.

Many hereditary cancer susceptibility genes, such as breast cancer 1 and 2 (BRCA1/2) and the group of DNA mismatch repair genes, have a known function in the DNA repair. Incomplete functioning of DNA repair appears to render somatic cells highly susceptible to carcinogenetic noxae and spontaneous DNA mutations, leading to an accumulation of genetic damage and ultimately transformation. Other susceptibility genes involving impaired DNA repair lead to cancer-prone syndromes such as xeroderma pigmentosa, Bloom's disease and hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome. Yet, there are inherited susceptibility genes having no direct function in DNA repair, but still showing an au-

tosomal dominant familial pattern. Von-Hippel-Lindau syndrome is a dominantly inherited hereditary cancer syndrome predisposing to a variety of malignant and benign tumors of the eye, brain, spinal cord, kidney, pancreas and adrenal glands. Other inherited cancer syndromes include ataxia telangiectasia, Li-Fraumeni syndrome, retinoblastoma, Wilms' tumor, familial adenomatous polyposis, multiple endocrine neoplasia 1 and 2, just to mention a few.

The hereditary mutations associated with cancer syndromes only have a big impact on the risk of a population if they are common. Thus, whilst mutations in the breast cancer susceptibility genes BRCA1 and BRCA2 are found in almost 10% of women with breast cancer, the PTCH1 gene mutation responsible for the Gorlin/basal nevus syndrome occurs in less than 1 per 50,000 of the population. However, it must be appreciated that the gene mutation frequencies vary considerably between populations, especially if the populations are isolated for geographical, religious or other reasons. Good examples in this context are BRCA2 mutations in Iceland and BRCA1/2 mutations among the Ashkenazi Jewish population. Inaccuracies in population estimates may bias clinical judgement and allocation of diagnostic resources (Hemminki et al. 2008).

Susceptibility to many diseases has been shown to be polygenic, with a multitude of low-penetrance common polymorphisms contributing to the risk of developing disease. These complex trait genes may contribute significantly to risk estimations of certain cancers. Therefore, it is useful to quantify the relative importance of known genes in the burden of disease by using the population attributable fraction (PAF) that states the contribution of the studied gene to disease aetiology, independent of the environmental or other genetic factors that may interact with the gene in question (Hemminki and Bermejo 2007). New approaches, such as genome-wide association studies (GWAS) using single nucleotide polymorphism (SNP) arrays, have provided tools to map and potentially identify some of the low-penetrance hereditary cancer-susceptibility genes. Future developments here will require large-scale multinational collaborations, similar to those conducted on breast cancer (Easton et al. 2007).

1.4 Oncogenes

Oncogenes are genes that, through the action of the proteins they encode, cause cancer when transcribed (Table 1.1). Oncogenes arise through the mutation of normal cellular genes with regulatory activities called proto-oncogenes. Recent data indicate that small RNA molecules called microRNAs (miRNAs) may control the expression of proto-oncogenes and that mutations in these may lead to oncogene activation (see Sect. MicroRNAs in human cancer) (Wiemer 2007; Negrini et al. 2007).

The first evidence for the existence of oncogenes was provided by the study of viral oncogenesis. In 1910, Peyton Rous prepared cell-free filtrates from sarcomas arising in chickens. Injection of the filtrate into other chickens resulted in the development of the same tumors in the recipient birds (Vogt 1996). The aetiological agent was identified as an avian RNA virus and subsequently named Rous sarcoma virus (RSV). Comparisons between the genomes of oncogenic and non-oncogenic RNA viruses quickly established that the oncogenic genomes uniquely harboured specific cancer-inducing genes. This led to the discovery of the first oncogene, the *src* gene in RSV (*v-src*). Its cellular homologue, *c-src*, was identified soon after, leading to the realisation that the viral oncogene was in fact a derivative of the cellular oncogene that had in an unknown manner, presumably during viral retrotransposition or during viral genome replication, been integrated into the viral genome and subsequently underwent rapid molecular evolution to acquire transforming potential. The final confirmation of the tumor-inducing role of oncogenes came from cell transfection studies, where genomic DNA from tumor cells containing active oncogenes was shown to be capable of transferring the malignant phenotype into recipient cells.

Studies with animal viruses have been essential in elucidating how the activation of oncogenes takes place and leads to cellular carcinogenesis. Even if our knowledge of human viruses causing cancer is based on in vitro studies and epidemiological data, it is reasonable to assume that transformation mechanisms in humans are closely related to those in animals. Some human pathogenic viruses causing cancer are listed in Table 1.2.

A typical example of a proto-oncogene translocation is the membrane tyrosine kinase receptor RET [see review (Santoro et al. 2004)]. The outer membrane part consists of four cadherin-like domains; the inner membrane domain has the tyrosine kinase activity. The gene was discovered in 1985 and was found to be activated by a DNA rearrangement, a mechanism giving the gene its name (Rearranged during Transfection). RET protein has several tyrosine residues that are auto-phosphorylated. The phosphorylation of the tyrosine 905 is sug-