Tumorigenesis

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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,

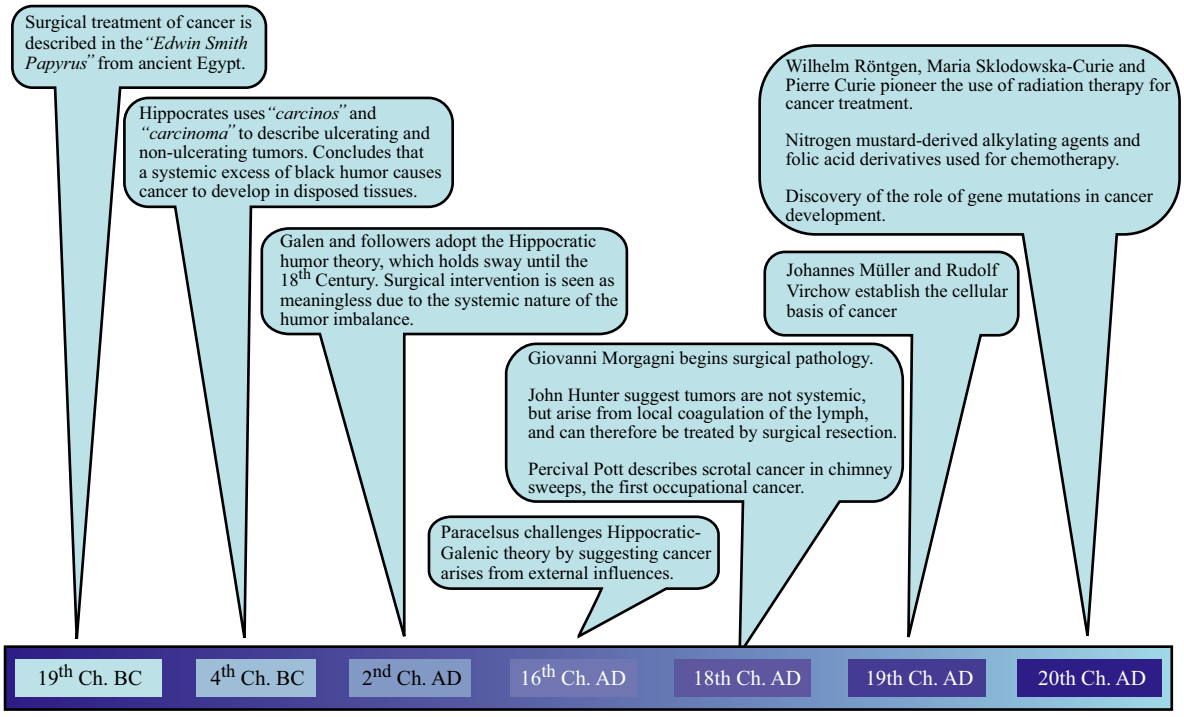

Fig. 1.1. Development of cancer biology over the centuries

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-

gested to act as a key in switching on the kinase activity. Other tyrosines serve as docking sites for signalling factors in their phosphorylated form. RET-mediated signalling pathways are shown in Fig. 1.2.

The RET gene, located in the long arm of chromosome 10 (10q11.2), is normally silent in thyrocytes. Due to a chromosomal inversion or translocation event taking place in a subpopulation of human papillary thyroid carcinomas (PTC), the tyrosine kinase-encoding part of the RET gene falls under the control of active promoter regions of several heterologous genes. The chromosomal rearrangements lead to a formation of chimeric RET/PTC oncoproteins that express constitutive tyrosine kinase activity. Different RET/PTC variants have been isolated that differ in the RET fusion partner. RET/PTC3, the fusion between RET and the RFG/Ncoa4 gene, is the most prevalent variant in radiation-associated paediatric PTCs. Data are accruing suggesting that the formation of RET/PTC oncogenes is causative in thyroid tumorigenesis. Thyroid follicular cells are transformed in vitro by RET/PTC. Furthermore, RET/PTC transgenic mice develop malignancy of the thyroid.

Table 1.1. Some oncogenes, their function and the pathways affected

Oncogene	Function	Pathway
Aurora A	Self-sufficiency growth signals	DNA repair
HPV-E6	Evading apoptosis	
MDM2	Evading apoptosis	
Abl	Self-sufficiency growth signals	Cell cycle control
CDK2	Self-sufficiency growth signals	
CDK4	Self-sufficiency growth signals	
Cyclin D	Self-sufficiency growth signals	
Cyclin E	Self-sufficiency growth signals	
HPV-E7	Self-sufficiency growth signals	
Gli	Evading apoptosis; self-sufficiency growth signals	Hedgehog signalling
Hedgehog	Evading apoptosis; self-sufficiency growth signals	
Smo	Evading apoptosis; self-sufficiency growth signals	
Akt	Evading apoptosis	Akt signalling
Bax	Evading apoptosis	
FKHR/FOXO	Evading apoptosis	
JAK	Evading apoptosis; self-sufficiency growth signals	
PI3K	Evading apoptosis	
B-Raf	Self-sufficiency growth signals	Ras signalling
Fos/Jun	Evading apoptosis; self-sufficiency growth signals	
ILK	Self-sufficiency growth signals; tissue invasion and metastasis	
Ras	Self-sufficiency growth signals	
RTKs	Evading apoptosis; self-sufficiency growth signals Tissue invasion and metastasis; sustained angiogenesis	
β-catenin	Self-sufficiency growth signals	Wnt signalling
RAR	Self-sufficiency growth signals	
SOX	Self-sufficiency growth signals	
Wnt1	Self-sufficiency growth signals	
Myc	Self-sufficiency growth signals	TGFβ signalling
Fas	Evading apoptosis	Death receptor
Notch	Evading apoptosis	Notch signalling
Gα	Self-sufficiency growth signals	GPCR signalling
GPCR	Self-sufficiency growth signals	

Table 1.2. Human viruses involved in cancer development

Virus	Non-tumor diseases	Tumor caused by infection
Human immunodeficiency virus	Acquired immune deficiency syndrome	Kaposi's sarcoma Non-Hodgkin's lymphoma Cervical cancer
Human papillomavirus	Warts	Cervical carcinoma Head and neck cancer
Hepatitis B	Hepatitis, liver cirrhosis	Liver cancer
Hepatitis C virus	Hepatitis, liver cirrhosis	Liver cancer
Epstein-Barr virus	Infectious mononucleosis	Burkitt's lymphoma Non-Hodgkin's lymphoma Hodgkin's disease Nasopharyngeal carcinoma
Human herpes virus 8	Castleman's disease	Kaposi's sarcoma Body cavity lymphoma
Human thymus-derived-cell leukaemia/ lymphoma virus-1	Tropical spastic paraparesis	Adult T cell leukaemia

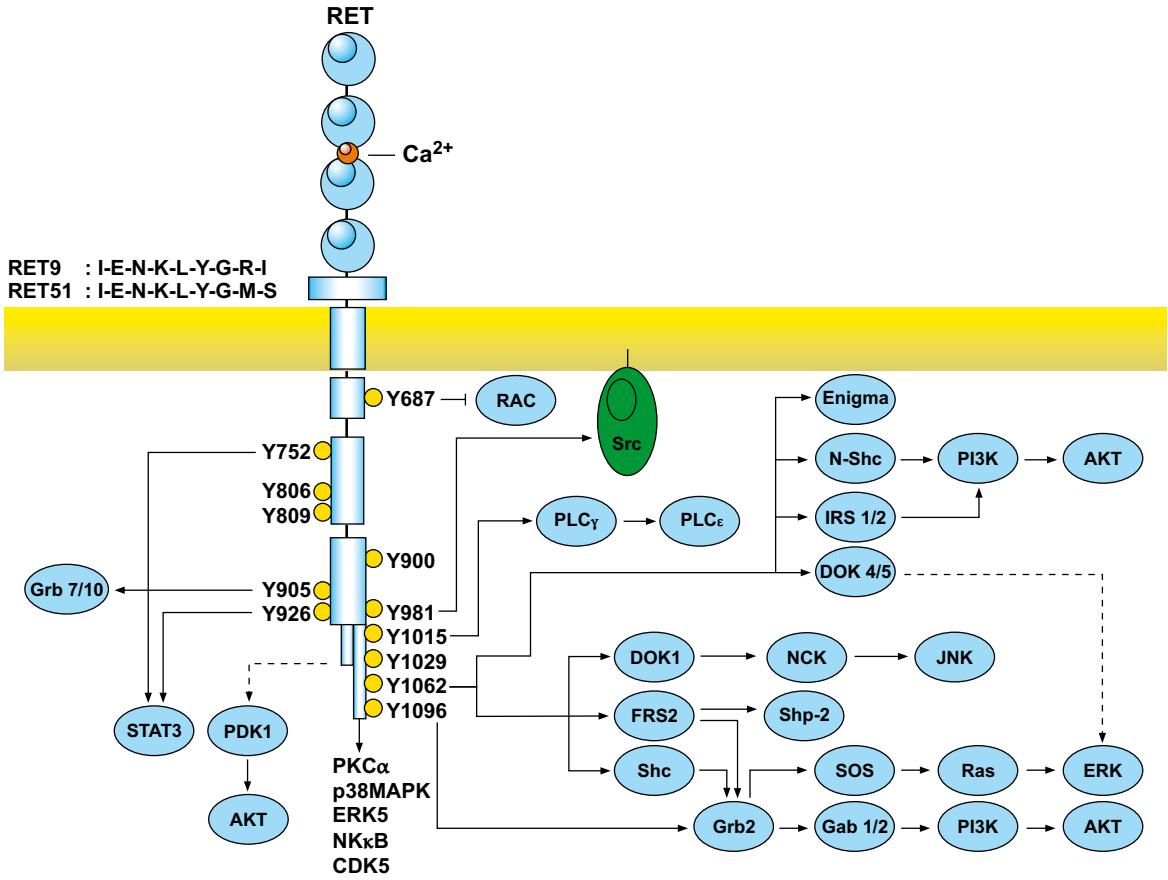

Fig. 1.2. The network of RET-mediated signalling events. RET auto-phosphorylation sites are shown with their direct targets. *Dotted lines* indicate pathways not yet fully elucidated. The amino acid sequences of RET9 and RET51 at the point in which they start to diverge at glycine 1063 are shown. With the courtesy of Dr. Massimo Santoro

1.5 Tumor Suppressor Genes

Our knowledge of tumor suppressor genes comes from seminal studies on the familial tumor syndrome retinoblastoma. Analysis of the frequency and age of onset of the disease in affected children revealed that bilateral disease had a much earlier onset than unilateral disease. The bilateral form of the disease is inherited by a germ-line mutation and is therefore present in all tissues, including both retinas, whereas the unilateral disease is due to a locally restricted somatic mutation affecting one eye only. To explain the earlier age of onset of the bilateral disease, it was proposed by Knudson (1996) that there must be a second event (subsequently proven to be loss of the remaining wild-type allele) that occurs earlier in the inherited form and later in the sporadic form. This two-hit model of inactivation of a tumor suppressor gene has remained a mainstay of our understanding of tumor suppressor gene inactivation. Inherited or sporadic mutation of one copy of the suppressor gene is postulated to confer a selection advantage to the cell clone, which through an undefined mechanism inactivates the remaining tumor suppressor allele. Many varieties of processes have been shown to

Table 1.3. Some tumor suppressors, their function and the pathways affected

Tumor suppressor	Function	Pathway
ARF	Self-sufficiency growth signals	DNA repair
ATM/ATR	Insensitivity to anti-growth signals	
BRCA1	Self-sufficiency growth signals; insensitivity to anti-growth signals	
Chk1	Insensitivity to anti-growth signals	
Chk2	Insensitivity to anti-growth signals	
DNA-PK	Insensitivity to anti-growth signals	
FANCD2	Insensitivity to anti-growth signals	
HIPK2	Evading apoptosis; self-sufficiency growth signals	
NBS1	Insensitivity to anti-growth signals	
P53	Evading apoptosis; insensitivity to anti-growth signals	
P15	Self-sufficiency growth signals	Cell cycle control
P16	Self-sufficiency growth signals	
Rb	Self-sufficiency growth signals	
Ptch	Evading apoptosis; self-sufficiency growth signals	Hedgehog signalling
Su(Fu)	Evading apoptosis; self-sufficiency growth signals	
Bcl-2	Evading apoptosis	Akt signalling
LKB1	Self-sufficiency growth signals	
PTEN	Evading apoptosis	
TSC1/TSC2	Self-sufficiency growth signals	
Integrin	Tissue invasion and metastasis	Ras signalling
NF1	Self-sufficiency growth signals	
VHL	Sustained angiogenesis	
APC	Self-sufficiency growth signals	Wnt signalling
Axin	Self-sufficiency growth signals	
α-catenin	Tissue invasion and metastasis	
E-cadherin	Self-sufficiency growth signals; insensitivity to anti-growth signals Insensitivity to anti-growth signals	
Wnt5A	Self-sufficiency growth signals	
BMPR	Insensitivity to anti-growth signals	TGFβ signalling
Smad2/3	Insensitivity to anti-growth signals	
Smad4	Insensitivity to anti-growth signals	
TGFβ R	Insensitivity to anti-growth signals	

be responsible for loss of the second allele (second hit), including copying the inactive mutant allele into the locus of the wild-type allele, interstitial deletion of the wild type allele, deletion of a chromosomal fragment or the entire chromosomal arm containing the allele. Inconveniently, a number of suppressor gene loci do not show loss of both alleles, leading to a number of models of how these non-classical suppressor genes are involved in cancer. Ideas range from inactivation of the second allele through epigenetic mechanisms, the presence of hypomorphic alleles at the remaining wild-type locus, gene dosage effects, etc. In all probability, each model may have its validity in explaining the tumor suppressor inactivation of a specific gene in a specific tumor type.

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).

Since the pioneering work by Knudson in the early 1970s, a correlation between mutated tumor suppressor genes and different cancers has been found in several cases, such as BRCA1 (cancers of breast, ovary, colon and prostate), BRCA2 (cancers of breast, ovary, pancreas and prostate), CDK4 (melanoma) and PMS1 and PMS2 (colorectal cancer), just to mention a few. Representative tumor suppressor genes, their functions and the pathways affected are listed in Table 1.3.

In addition to an increased risk of cancer, individuals with germ-line mutations in tumor suppressor genes frequently show an increased susceptibility to radiation, with Li-Fraumeni (TP53), Gorlin (PTCH1) and retinoblastoma (RB1) syndromes being frequently encountered (Evans et al. 2006). The majority of reported cases with radiation-induced cancers carry mutations in RB1, with almost 40% of affected individuals developing radiotherapy-associated tumors compared to a sporadic rate of 20% in non-radiotherapy cases (Aerts et al. 2004; Kleinerman et al. 2005).

The retinoblastoma protein, Rb1, is a tumor suppressor found to be dysfunctional in several human cancers (Murphree and Benedict 1984). The gene RB1 encodes a factor that controls the progression of the cell cycle through the G1 phase and into S phase. The function of Rb1 depends on its phosphorylation state; Rb can actively inhibit cell cycle progression in its dephosphorylated form by binding and thereby inhibiting transcription factors of the E2F family (Korenjak and Brehm 2005). Rb-E2F complex stalls the cell cycle progression and allows repair of DNA damage before the cell enters the S-phase. Rb is initially phosphorylated by cyclin D1/CDK4/6 (Fig. 1.3), followed by additional phosphorylation by cyclin E/CDK2, allowing the cell to enter the S-phase. Rb1 remains phosphorylated throughout S, G2 and M phases and is again dephosphorylated near the end of G1 phase, allowing it to bind E2F (Vietri et al. 2006).

1.6 MicroRNAs in Human Cancer

MicroRNAs (miRNAs) are evolutionary conserved small non-coding RNAs, ranging in length from 16 and 29 nucleotides. The miRNAs are postulated to form an endogenous system to regulate and coordinate the expression of genes on a post-transcriptional level (Wiemer 2007; Negrini et al. 2007). They are able to bind complementary sequences in target messenger RNAs (mRNAs) and thus prevent their translation. Each miRNA may potentially target several hundreds different mRNA molecules, suggesting they may exert a one-step control over cellular processes (Lewis et al. 2005).

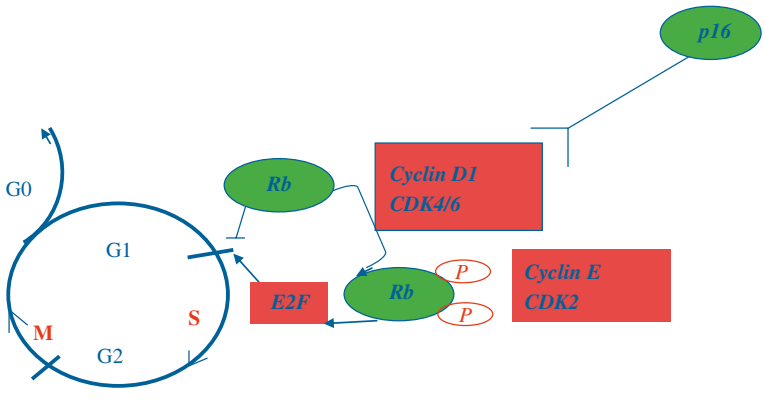

Fig. 1.3. The regulation of the cell cycle by the phosphorylated and non-phosphorylated forms of Rb1 protein. *Green ovals* represent proteins with tumor suppressor funktion, *red squares* proteins acting as oncogenes

The exact mechanism of the translational "silencing" is not known, but recently the target mRNAs were found to be sequestered in the so-called processing bodies (P bodies) distant from the translating ribosomes (Coller and Parker 2005; Liu et al. 2005; Sen and Blau 2005).

At the moment, more than 4,000 different miRNAs are identified or predicted in the genomes of viruses, plants and animals, of which some 700 may occur in man (Griffiths-Jones et al. 2006). Some mammalian miRNAs are located within gene introns and appear to be transcribed within the primary transcript, only to be released during RNA processing (Shivdasani 2006).

In recent years, miRNAs have been shown to influence a variety of cellular processes of key importance, including cellular differentiation and maintenance of a differentiation state, developmental timing, proliferation and apoptosis (Alvarez-Garcia and Miska 2005; Zhang et al. 2007). Since deregulated cell death and proliferation are hallmarks of many types of carcinomas, it is not surprising that, on the one hand, alterations in miRNA may lead to carcinogenesis, and, on the other hand, many miRNAs are found to be abnormally expressed in clinical cancer samples.

The first study showing involvement of miRNA in human cancer was done by Calin et al. (2002). In search of a tumor suppressor gene in chronic lymphocytic leukaemia (CLL) cases, they found that the smallest common lesion of a 30-kb region located at chromosome 13q14 coded for two miRNAs, miR15 and miR16. Furthermore, both genes were found to be deleted or downregulated in a majority (approximately 68%) of CLL cases. The discovery of a germ-line point mutation in two CLL patients that resulted in downregulation of both miRNAs and the induction of apoptosis by miR15 and miR16 by negatively regulating anti-apoptotic oncogene BCL2 in the leukaemic cell line MEG-01 supported the putative tumor suppressor role of these miRNAs (Calin et al. 2005; Cimmino et al. 2005).

MiRNAs may also act in an oncogene-like manner. The amplification of the miRNA gene cluster miR-17-92 on chromosome 13 in human B-cell lymphomas leads to upregulation of several miRNAs that together with MYC oncogene accelerate tumor development (He et al. 2005). Transcription of this cluster is induced by MYC itself. Similarly, overexpression of miR-155 in B-lymphocytes of transgenic mice leads to pre-leukaemic pre-B cell polyclonal expansion followed by B-cell malignancy (Costinean et al. 2006).

Considering how rapidly data have been accruing in the last years, it is reasonable to believe that the next decade will bring new insights about the role of miRNAs in carcinogenesis and their therapeutic tools.

1.7 Lifestyle, Environmental and Occupational Factors Causing Cancer

Known carcinogens include a number of different substances, mixtures and exposure circumstances 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. Based on epidemiological data and biological data from both human and animal material, the International Agency for Research on Cancer (IARC) has classified agents, mixtures and exposures into five categories (IARC): Category 1: carcinogenic to humans; category 2A: probably carcinogenic to humans; category 2B: possibly carcinogenic to humans; category 3: not classifiable as to carcinogenicity in humans; category 4: probably not carcinogenic to humans.

Some examples of different types of category 1 carcinogens include gamma radiation (lung, liver, skeletal and other solid cancers) and underground mining with exposure to radon (lung cancer); arsenic compounds (cancers of skin, lung, bladder and liver); aflatoxin B1 produced by the fungus *Aspergillus flavus* growing on grains and nuts (liver cancer); various viruses such as hepatitis B and C (liver cancer), Epstein-Barr virus (Burkitt's lymphoma, non-Hodgkin's lymphoma, Hodgkin's disease) and human papilloma virus (cervical cancer); and bacteria, such as *Helicobacter pylori* (gastric cancer), just to mention a few.

To what extent populations come into contact with different carcinogens depends largely on cultural and socioeconomic factors such as diet, and tobacco and alcohol consumption. Populations of less-developed countries are to a much greater extent exposed to indoor pollution caused by cooking fumes and solid heating fuels than those in developed countries (Lissowska et al. 2005). On the other hand, the broiling and barbecuing meat at high temperatures typical for western civilisations lead to the formation of polycyclic aromatic hydrocarbons (PAH) and tars that are potent carcinogens (Felton et al. 1997; Sugimura et al. 2004). However, apart from consumption of alcohol or aflatoxin-contaminated food (IARC 1993), no single dietary factor can be pinpointed as a definite cause of cancer. More importantly, lifestyle factors leading to obesity and increased tobacco and alcohol consumption are probably causing more cancer cases, either directly or as co-factors, than any single factor alone (Doll et al. 2005; Peto 2001).

The carcinogenic effect of cigarette smoking is by far the most important discovery of modern epidemiology

(IARC 1986). The steep rise in the cigarette consumption among the Western European male population after the First World War and among the North American male population after the Second World War was tracked by increase in carcinomas of the lung (Peto 2001).

There are about a dozen occupational exposure situations known to increase the risk of cancer, mostly carcinomas of the lung. In many cases the carcinogens are in airborne complex mixtures with other carcinogens and co-factors. Especially in underground mining, the workers may be heavily exposed to several carcinogens, such as coal, dust, asbestos, radon and arsenic (Taylor et al. 1989; Tapio and Grosche 2006; Grosche et al. 2006; Wichmann et al. 2005; Liu et al. 2002).

Asbestos is a naturally occurring fibrous silicate that has been widely used for insulation. Epidemiological data show a strong correlation between asbestos and pleural and peritoneal mesotheliomas as well as lung cancer (Boffetta 2007; Becklake et al. 2007). Due to the long latency of about 30 years and more, incidence rates are still rising, and it is estimated that occupational exposure prior to 1980 will eventually cause 250,000 cases of mesothelioma and the same amount of lung cancer cases in Western Europe (Peto et al. 1999). According to the WHO, 5% of the European population are environmentally exposed to asbestos, leading annually to approximately 1,500 additional cases of mesothelioma and lung cancer (WHO 1987; Boffetta and Nyberg 2003).

Arsenic is a widely distributed semi-metallic compound that causes several types of cancer due to both environmental and occupational exposure situations (Tapio and Grosche 2006). The primary route of environmental exposure is drinking water contaminated with inorganic arsenic. Contrarily to the organic arsenic compounds frequently present in seafood, inorganic arsenic, especially in its trivalent forms, is a group 1 carcinogen (IARC 2004). Inhalation of arsenic-contaminated dust is a common problem in tin, gold and uranium mines (Chen and Chen 2002; Taylor et al. 1989; Kusiak et al. 1991; Grosche et al. 2006). Whilst inhalation of airborne arsenic in glass and copper smelters or arsenic-contaminated dust in mines causes mostly lung cancer, arsenic in drinking water increases additionally the risk of bladder, liver, skin and kidney cancers. Both inhalation and ingestion of inorganic arsenic compounds are correlated with the increased occurrence of squamous cell carcinomas of the lung and skin in comparison to other types of lung and skin carcinomas (Tapio and Grosche 2006).

In China, Bangladesh and India, millions of people have been exposed to arsenic-contaminated drinking water since the 1980s (Table 1.4). Formerly, shallow well water or surface water was used in households, causing other health problems. The effort to improve the quality of drinking water by drilling deeper wells led to the unanticipated opposite effect by increasing the amount of arsenic in the water leaking from the surrounding soil.

Table 1.4. Countries with arsenic-contaminated drinking water (from Tapio and Grosche 2006)

Country	Number of people affected	Arsenic concentration (μg/l)
Bangladesh	50–75 Million	<10–>1,000
West Bengale (India)	>6 Million	3–3,700
China	>2 Million	50–2,000
Taiwan	120,000 (1982)	200–2,500
Thailand	n.d.	1–5,114
Vietnam	11 Million	1–3,050
Mexico	400,000	8–624
Chile	250,000	470–770
Argentina	2 Million	>100
United States	350,000	1–1,160
Finland	10,000	17–980
Hungary	n.d.	1–174

In large areas of endemic arsenic poisoning, the rate of malignancies is expected to explode within the next decades.

1.8 Cancer Stem Cell Hypothesis and Microenvironment

Stem cells are pluripotent undifferentiated cells capable of undergoing a self-renewing cell division in contrast to embryonic stem cells that are omnipotent. The asymmetric division of a stem cell, by definition, yields one daughter cell that can differentiate along multiple lineages and a daughter stem cell with all the properties of the parental stem cell. A spectrum of cells with varying degrees of stemness is recognised by phenotypic markers. These cells are presumed to represent the second or third generation of stem cells that have undergone some preliminary commitment to one or more of the tissue lineages. Thus, a mesenchymal stem cell may differentiate to produce adipocytes, fibroblasts, osteoblasts and a host of other mesenchymal cells, but it is committed to the mesenchymal lineage.

In 1926, Bailey and Cushing proposed that cancer was initiated and maintained by a subpopulation of transformed precursor cells. However, it was not until recently that Dick (2005) and co-workers showed that only a few (0.1–1%) of the tumor cells present within an acute myeloid leukaemia (AML) sample had the capacity to initiate AML growth after transplantation into NOD/SCID mice. Since then, small populations of cells with self-renewing capacity have been isolated from most leukaemias, solid cancers such as medulloblastoma and glioblastoma, as well as carcinomas of different organs. These putative cancer stem cells are defined as cancer cells with stem-like properties, such as the ability to remain quiescent for long periods of time and the capacity for asymmetric cell division giving rise to one cancer stem cell and one differentiated progeny. However, they differ from normal stem cells by demonstrating unregulated proliferation, probably due to acquired gene mutations that render them less responsive to negative growth signals or to the loss of contact inhibition and gap junction intercellular communication. They display the same cell surface markers as their normal tissue counterparts, allowing their isolation and enrichment.

The definition of cancer stem cells directly implies that a cancer treatment can only be successful if all cancer stem cells are killed. A subset of cancer stem cells expresses multidrug resistance transporters ABCB1 and ABCG2 (Mimeault and Batra 2007); others express constitutively vascular endothelial growth factor receptors (VEGFR2) and seem to be the source of intrinsic vasculature building for the tumor (Shen et al. 2008). Studies with glioblastoma and breast cancer stem cells indicate an increased radioresistance due to a more efficient DNA damage repair compared to non-stem cancer cells (Baumann et al. 2008). The development of approaches to radiosensitise tumor stem cells remains an important future challenge.

Although many fruitful studies on cancer biology have been performed in monotypic cell culture, the basic structural unit of living tissues remains a highly complex three-dimensional mixture of cell types. In 1959, Letterer defined the morphology of this complex mixture as a histion; more recently, the term microenvironment has been used. It is important to note that the microenvironment includes not only different cell types, such as fibroblasts, endothelial cells, tissue macrophages, leucocytes, nerve cells, etc., but also extracellular matrix, serum and lymph proteins, and a whole host of locally- and systematically-acting secreted molecules. Within the microenvironment, tumors develop and interact with the different components. It seems unwise to assume that tumor stem cells are immune from the influence of this microenvironment (Kenny et al. 2007).

1.9 Radiation-Induced Cancers

Ionising radiation is an effective carcinogen, causing malignant transformation of many different tissues. The shape of the dose-response relationship for cancer induction is currently assumed to be best represented by a linear no-threshold relationship. This also describes the dose response observed for the accumulation of damage to cellular macromolecules, in particular DNA. Although not universally accepted, it is considered that a failure to repair DNA damage leads to the permanent accumulation of gene mutations in irradiated tissues that then lead to alterations in the regulatory pathways described above. Alternative views give more weight to non-targeted effects of radiation damage, including local inflammatory and stress responses, which are postulated to lead to more global changes in mutational activity characterised by genomic instability.

Evidence for a direct, targeted, mutational event in radiation-induced cancers is lacking, even for alpha-radiation, which would be expected to induce character-

istic large deletions in critical genes, which should then be present in all progeny cells.

A number of studies have reported either specific gene alterations (e.g. RET/PTC3 translocations in radiation-induced thyroid cancer, AML-ETO alterations in radiation-associated myeloid leukaemia) or a specific profile of gene expression changes (e.g. in radiation-induced osteosarcoma and papillary thyroid cancer). However, the specificity of these changes may reflect the histopathological uniqueness of the radiation-induced tumors, which suggests that they may be derived from different progenitor cells than those giving rise to sporadic cancers in these tissues. An additional complication is that many radiation-induced cancers arise in genetically predisposed individuals who have inherited a germ-line mutation (e.g. in the RB1 gene). The mechanisms behind the development of therapy-associated cancers in such an individual may well be quite different from those in sporadic cancers.

1.10 Conclusions

The underlying molecular mechanism responsible for the development of a tumor cell may vary (e.g. inactivation of a tumor suppressor gene by a virally encoded protein, inheritance of a germ-line mutation or sporadic point mutation of an oncogene). Nevertheless, all of the mutational events target a common set of regulatory nodes within the cell, such as the cell cycle checkpoints, growth factor independence and prevention of apoptosis. The wide spectrum of genetic alterations, even within one tumor type, reflects the multiple points at which key processes may be subverted and camouflages a much more simple biological process involving only a set of critical processes.

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. In all three situations, the role of ionising radiation is, at best, poorly understood, and harnessing them for therapeutic purposes requires that considerable effort be expended to define their interaction with radiation.

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