In recent decades we have been given insight into the process that transforms a normal cell into a malignant cancer cell. It has been recognised that malignant transformation occurs through successive mutations in specific cellular genes, leading to the activation of oncogenes and inactivation of tumor suppressor genes. The further study of these genes has generated much of its excitement from the convergence of experiments addressing the genetic basis of cancer, together with cellular pathways that normally control important cellular regulatory programmes. In the present review the context in which oncogenes and tumor suppressor genes normally function as key regulators of physiological processes such as proliferation, cell death/apoptosis, differentiation and senescence will be described, as well as how these cellular programmes become deregulated in cancer due to mutations.

Keywords: oncogenes; tumor suppressor genes; proliferation; apoptosis; differentiation; senescence

Introduction

It is today clear that cancer is caused by specific mutations in specific key regulatory genes. Since the discovery of the first such genes some 30 years ago, we have seen remarkable progress in the definition of genetic lesions in malignancy, and furthermore our general understanding of cell biology has made us able to comprehend how these cancer genes normally function in the control of the non-malignant cell, and how this relates to the ability of these genes to cause malignant transformation when mutated.

In normal tissues of multicellular organisms the function of the single cell is tightly controlled due to inner and surrounding constraints, to maintain tissue homeostasis and function. During malignant transformation the cancer cell will gradually become more autonomous, and develop into an 'asocial citizen' of its tissue, growing in an uncontrolled manner at the expense of the function of the normal tissue. More specifically, the cancer cell has obtained relaxed control of several normal cell regulatory mechanisms. The most obvious such function is proliferative control. In many types of malignancies (but not all), the tumor cells divide at a higher rate, often irrespective of the extracellular growth signals that usually control cell growth. Although obvious today, it was not until recently that it was realised that tissue growth is not only regulated by the rate of cell division, but that the rate of cell death or apoptosis is another key factor in tissue homeostasis. With this came the understanding that malignancy may also develop through reduced cell death rate. Furthermore, a common feature of cancer cells is their inability to differentiate terminally, which in normal tissues would lead to cessation of proliferation. Cell cultures derived from human tumors often divide indefinitely; furthermore, the number of population doublings a cell has to go through in order to become a clinically detectable tumor is very large. This is in sharp contrast to non-transformed cells which, with few exceptions, have a limited lifespan and will, after a defined number of population doublings, enter a non-
replicative but metabolically active state termed senescence. This has led to the hypothesis that cellular senescence is a tumor suppressor mechanism which is abrogated in malignancy.

As previously mentioned a number of genes that are specifically mutated in malignant cell have been defined to date. They can generally be divided into two groups: oncogenes and tumor suppressor genes. Oncogenes take part in malignant transformation due to activating mutations such as amplification, small mutations or translocations. Due to their activating nature, mutations in these genes are usually dominant and only one allele of the gene needs to be affected. The function of tumor suppressor genes on the other hand seems to be to protect the normal cell from developing into a cancer cell, and a loss of their function leads to malignant transformation. As the majority of human genes are present in two copies in the genome, both alleles usually have to be inactivated for their tumor-suppressing activity to be lost. Recently a third category of cancer-related genes has been defined; namely the DNA repair genes. These are a group of genes that take part in the normal repair of DNA damage, and somatic or inherited loss of their function leads to an increased frequency of secondary mutations in oncogenes and tumor suppressor genes.

With the definition of the molecular background of a number of different inherited cancer syndromes, it has been shown that the increased cancer risk in these families may often be due to the inheritance of a mutated tumor suppressor gene.

The present review will not give a detailed list of known cancer-related genes, but will rather describe the normal context in which these genes regulate physiological processes such as proliferation, cell death/apoptosis, differentiation and senescence, and how they become deregulated in the malignant cell due to mutations in oncogenes and tumor suppressor genes. The review will not deal to any major extent with other factors that may be of importance in the malignant process, such as the mechanism behind the acquisition of metastasising potential or the interaction of tumor cells with surrounding stroma and blood vessels.

**Proliferation**

Our understanding of the regulation of eukaryotic cell division took a quantum leap some ten years ago, when it was realised that cell cycle progression is governed by sequential formation and activation of enzyme complexes consisting of a family of related cyclin-depend-
mutations in the very key cell cycle regulatory proteins such as overproduction of cyclin D1 or cdk4 or inactivation of the cki p16. The inactivation of p16 seems to occur at high frequencies in a wide variety of malignancies such as leukaemias, melanomas, pancreatic cancer and bladder cancer.8 Malignant cells may also have mutations in the more downstream effector molecules, such as inactivation of the retinoblastoma gene or overproduction of E2F responsive genes such as c-myc. As delineated above, cancer cells commonly have mutations at some level of the molecular machin-

ergy that regulates G₁ progression into S phase, explaining increased and uncontrolled proliferation in malignancy (Figure 1).

Apoptosis
It is not until recently that the major importance of tightly regulated cell death in tissue homeostasis has been duly recognised. Apoptosis is the descriptive name given to the process of physiological programmed cell death in vertebrates (for a review see Ref. 2). During apoptosis a cell activates an intrinsic suicide machinery that results in cell death: its surface membrane begins to bleb, the cell shrinks, the chromatin becomes condensed and cleaved, and eventually the whole cell fragments into membrane-bound vesicles that are rapidly ingested by neighbouring cells. Apoptosis has been shown to be important in a number of physiological processes such as embryonic development, immune regulation and tissue homeostasis. Recently we have gained important knowledge in the molecular regulation of the apoptotic process.

A number of stimuli have the capability of activating the apoptotic programme. These stimuli include both extracellular factors, such as the FAS antigen, TNF, interferons and matrix attachments as well as intracellular events, as for example DNA damage (Figure 2).2

However, not every cell that is exposed to an apoptotic signal undergoes apoptosis, which demonstrates the importance of intracellular modulators regulating the sensitivity of the cell. The Bcl2 family of proteins are the best known rheostats in regulating the cellular sensitivity to apoptosis.9 The family members either promote cell survival (eg Bcl2) or augment programmed cell death (eg Bax). It is believed that the agonistic and antagonistic proteins of the Bcl2 family interact through hetero- or homodimerisation, and the relative protein amounts of pro- and anti-apoptotic Bcl2 family members will determined whether an apoptotic signal will result in cell death or not (Figure 2).

Recently, an emerging family of cysteine proteases have been found to be essential downstream effectors in the actual killing of the apoptotic cell. In this family the nematode C. elegans protease Ced3 and its vertebrate homologue interleukin-β converting enzyme (ICE) are the prototype cysteine proteases.10 All the members in the ICE/Ced3 protease family share some features:

1. They all induce apoptosis when overexpressed.
Recent studies have revealed that the Bcl-2 oncogene functions in preventing apoptosis instead of promoting proliferation, not only establishing a new class of oncogenes, but also revealing the fact that malignant cells often exhibit decreased sensitivity to apoptotic signals leading to prolonged survival. Furthermore, it seems that most chemotherapeutic agents and radiotherapy utilised in anticancer treatment act through induction of apoptosis in the malignant cells. It has also been realised that the p53 tumor suppressor gene is involved in the apoptotic response to several types of DNA-damaging agents, including chemotherapy and radiotherapy. The p53 gene is the hitherto most commonly mutated gene in human cancer, as aberrations in this gene is estimated to occur in more than 50% of all tumors. Tumor cells with p53 mutation thus have a reduced susceptibility to apoptosis induced by several stimuli, including agents used in anticancer therapy. Several other traditional oncogenes and tumor suppressor genes, such as c-myc and the retinoblastoma gene, have also been implicated in regulating cell death, even though the mechanism behind this is less clear.

**Differentiation**

The ability of cells to differentiate in an orderly and controlled manner is of major importance for multicellular organisms, where all specialised cells are derived from a single totipotent cell. In comparison with our comprehension of cellular proliferation and apoptosis, our understanding of the differentiation process is less clear, possibly reflecting a greater complexity in its regulation and a larger variability between tissues. One process that has been studied in some detail is haematopoiesis and will therefore be further discussed as an example in this context.

All types of mature specialised blood cells are believed to originate from a small subset of ancestor cells. During haematopoiesis immature multipotent stem cells undergo progressive restriction of lineage potential to give rise to mature, terminally differentiated functional blood cells, which eventually undergo programmed cell death (Figure 3). This process is regulated by the intricate cooperation between exogenous and endogenous molecules. The exogenous molecules include different cytokines,
whose cellular response is activated by binding to specific receptors. These receptors transform the cytokine message into cellular signals that ultimately result in altered DNA transcription, thus leading to the activation of different genetic programmes and progressive differentiation. At the centre of this transcriptional regulation induced by the external factors reside endogenous cell-specific proteins known as transcription factors. These proteins bind to short stretches of DNA in a sequence-specific manner and thus alter the transcription of specific genes in a positive or negative manner. Some of these transcription factors have been found to coordinate the expression of many different genes involved in lineage specific genetic programmes of differentiation, and are thus thought of as master or key transcriptional regulators (Figure 3).

In leukaemia as well as other malignancies, the malignant cells commonly have lost the capacity to differentiate, and become frozen at a certain stage of differentiation. The pathomolecular background to this is not entirely known, but in acute leukaemias a recurrent theme of genetic alteration is the occurrence of translocations involving known differentiation regulating transcription factors (Figure 3). These translocations usually lead to the formation of chimeric proteins which either abrogate the function of the transcription factor or lead to activation at an inappropriate time-point, presumably perturbing the normal differentiation programme of the affected cell. It has furthermore been suggested that abrogation of the differentiation blockage in malignant cells could lead to reversion into a non or less malignant state. In different in vitro systems, mainly utilising leukaemic cell lines, a wide variety of compounds have indeed demonstrated differentiating activity with the induction of mature non-proliferating cells. Despite the progress with in vitro systems, little has been achieved in vivo. To date, only patients with acute promyelocytic leukaemia have come to benefit from this approach. This disease is characterised by a balanced translocation between chromosomes 15 and 17, producing a chimeric protein fusing the retinoic acid receptor RARα to the transcription factor PML. In the majority of these patients treatment with retinoic acid leads to complete remission with the appearance of mature cells containing the t(15;17). However, retinoic acid treatment does not cure acute promyelocytic leukaemia, as the disease usually relapses in the absence of consolidating therapy.

**Senescence**

As described by Hayflick more than 30 years ago, human cells have a limited lifespan and will, after a defined number of population doublings, enter a non-replicative but metabolically active state termed senescence. During replicative senescence, cells arrest in the G1 phase of the cell cycle. With the discovery of a number of proteins normally regulating the cell cycle and G1 progression, some of the molecular events involved in replicative senescence have been identified. It has recently been demonstrated that senescent cells express increased levels of the previously mentioned ckis p16, p15, p21. The p16 and p15 proteins accumulate as a consequence of the increasing number of population doublings, eventually leading to senescence. Another feature shared by senescent cells is telomere shortening. The telomeres are repetitive sequences at the ends of chromosomes, and their shortening has been suggested as a candidate for a cell division 'counting' mechanism in normal somatic cells.
of higher organisms,\textsuperscript{16} as the telomere length decreases with increasing population doublings. It has been proposed that during successive rounds of replication, progressive loss of telomere length is sensed as DNA damage and causes cells to exit the cell cycle.

Senescence has been suggested to be an important tumor suppressive mechanism, and there is substantial evidence to support this idea.\textsuperscript{4} First, senescence prevents cells from acquiring the multiple mutations that are needed for malignant transformation. Indeed, many, if not most, malignant tumors contain cells that have an extended or indefinite division potential. During malignant transformation there seems to be a selection for cells that can bypass senescence. Secondly, several oncogenes, both cellular and viral, act at least in part by extending the replicative lifespan. Furthermore, the p16 and p15 ckis that have been shown to accumulate during normal senescence, are commonly inactivated in many human tumors, possibly allowing the malignant cells to escape the senescence programme.\textsuperscript{8} Malignant cells have often also acquired an ability to maintain telomere length through the expression of the enzyme telomerase which adds essential telomeric sequences to maintain the ends of chromosomes.\textsuperscript{16}

Other Deregulated Processes in Malignant Cells

Although it is not the scope of the present review to cover other functions that may be altered in malignant cells and their molecular background, one such area, metastasising potential, will be mentioned briefly. The majority of cancer-related deaths are not due to growth of the primary tumor, but rather because of metastasising disease. The molecular background of metastasis is still unclear, even though some mechanisms have been proposed, such as changes in cell adhesion molecules and expression of proteases.\textsuperscript{17}

 Alterations in the above mentioned oncogenes and tumor suppressor genes leading to transformation of cells exhibiting undifferentiated features, autonomous growth and decreased sensitivity to apoptotic signals, probably also create cells with an ability to reside in other environments than the original organ. This is also exemplified by a host of animal experiments, in which normal cells transfected with a limited number of oncogenes gain the ability to form tumors at various sites in the animal. Other important functions aiding in the malignant transformation is the ability of the malignant cells to interact with the surrounding stroma and vasculature, but these functions will not be further discussed.

Current Perspectives and Future Directions

During recent decades we have seen remarkable progress in our understanding of the events that transform a normal cell into a malignant cell. It is now clear that malignant transformation is caused by specific mutations in oncogenes and tumor suppressor genes. We are also starting to comprehend how these mutations lead to the malignant phenotype, as we begin to understand the way in which these genes interact with cellular programmes such as control of proliferation, apoptosis, differentiation and senescence, and the way that mutations in oncogenes and tumor suppressor genes lead to a relaxed control of these processes. To date, this increase in our basic knowledge of malignant transformation has unfortunately been of little help to the vast majority of patients with cancer. Hopefully, we are now starting to obtain the tools to utilise this knowledge in designing more efficient and specific anticancer therapies. The current understanding of the malignant process has, for example, formed the basis of a number of current clinical pilot studies, based on gene-therapeutic approaches. In the future we will probably be able to design chemicals and peptides that can specifically interfere with the functions that are dysregulated in malignant cells. Although no clear data concerning the beneficial effects of such novel treatments exist to date, some studies have shown promising results, giving clear hope for the future.

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