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Molecular oncology: prospects for cancer diagnosis and therapy



In industrialised countries mortality from cancer has remained almost unchanged in recent decades, whereas mortality from cardiovascular diseases has fallen considerably. If these trends continue, malignant diseases will become the leading cause of death in five to ten years time. In recent years the molecular causes of cancer have been studied in depth. Better understanding should lead to new approaches to the treatment of cancer.

Every tumour is different

Why is cancer so difficult to treat? Why is successful treatment so uncommon that despite heavy funding of cancer research, cancer is likely to become the leading cause of death in industrialised countries within a decade? The answer is that cancer is a complex disease that varies widely in terms of its causes, development, and progression.

At the molecular level, the problem of how to reduce cancer mortality is approached principally in the following three ways:

1. Identification of patients who have a genetic predisposition to develop cancer (detection of germline mutations in patients with increased familial incidence of cancer) and detection of genetic changes in somatic cells that favour degeneration to form cancer cells.
2. Early detection: the best method of combating cancer is by diagnosing it *early*. Early stages of cancer can generally be treated successfully, whereas advanced cancer is difficult to treat and has a poor prognosis.
3. Development of new molecular approaches that target the tumour cell or the tumour environment.

In order to explain why cancer is so difficult to diagnose and treat, a number of very general aspects of the disease must first be considered.

Tumour development: a multistage process

Tumours are generally clonal, i.e. they develop from a single cell. This cell acquires a series of mutations or epigenetic changes that influence certain cellular pathways and processes including signal transmission and DNA repair. Genetic or epigenetic changes that interfere with certain cellular processes are referred to here as 'pathway events'. These include changes in the base sequence of DNA and methylation¹ of DNA bases in promoter regions² of genes [1]. The occurrence of a pathway event in a normal cell can confer a growth advantage on the resulting cell clone. After expansion of the clone, another pathway event occurs in one of the cells of the clone, once again conferring a growth advantage on the affected cell, and so on. This model of tumour development via a series of pathway events in a specific sequence is

1 Attachment of methyl groups (-CH₃) to cysteine residues is known as methylation.

2 see glossary

Glossary

- Amplification:** An increase in the number of copies of a gene. In tumour cells this is commonly associated with a growth or survival advantage and can contribute to the development of resistance to chemotherapy.
- Deletion:** Loss of individual nucleotides of DNA, DNA fragments, or chromosome segments.
- Promoter methylation:** Methylation of the promoter region (the site on DNA at which transcription is initiated by binding of RNA polymerase).
- Polymorphism:** The existence in a significant proportion of a population of two or more alternative forms of a gene at the same locus of a chromosome.
- Transcription:** Synthesis of an RNA copy from a gene.
- Translation:** Synthesis of a polypeptide the composition of which is determined by the sequence of bases in messenger RNA (mRNA).
- Translocation:** Transfer of a chromosome segment to a non-homologous chromosome.

Meaning of abbreviations

- APC:** Gene that is altered in familial **A**denomatous **P**olyposis **C**oli.
- BRCA1 / BRCA2:** Genes that show certain mutations in hereditary forms of **B**reast **C**ancer.
- K-RAS:** RAS genes are oncogenes, i.e. genes which, if they show mutations or disturbed regulation, can play an active role in the development of malignant tumours.
- MEN 2:** **M**ultiple **E**ndocrine **N**eoplasia, type **2**: a condition characterised by the appearance of hormone-producing tumours in two or more organs.
- P53:** Gene that encodes phosphoprotein P53. Protein P53 acts as a tumour suppressor.
- RAD51:** The RAD51 protein that is encoded by the *RAD51* gene, plays a role in the repair of DNA double-strand breaks caused by, for example, irradiation. The name is derived from **R**ADiation.
- RET:** Oncogene that encodes a receptor-linked tyrosine kinase and is altered in many malignant thyroid tumours. The name is derived from **R**Earranged during **T**ransfection.

known as the multistage model of carcinogenesis [2]. It states that a tumour goes through a series of stages before becoming manifestly malignant. Transition from each stage to the next is associated with a specific new pathway event. Whereas promoter methylation generally inactivates cellular processes, mutations may, depending on their type, either activate or inactivate cellular processes.

The cellular processes that promote the clonal development of tumours have been described above all in terms of tumour-associated genetic lesions. There are solid grounds for believing that the normal rate of mutation is not sufficient to account for the sequential mutations that a cell undergoes during malignant transformation. In fact, processes that result in genomic instability are crucially important for the development of tumour cells. Other processes influence the number of cells by controlling proliferation, differentiation, or programmed cell death (apoptosis).

Specific processes can be essential for the development of certain tumour types, though tumours can also develop via alternative pathways. For example, inactivation of the *APC* signal pathway appears to be a necessary condition for spontaneous development of colorectal carcinomas, however, *K-RAS* mutations are present in only a third, and *P53* mutations in only around half, of these tumours (Fig. 1). Other pathway events

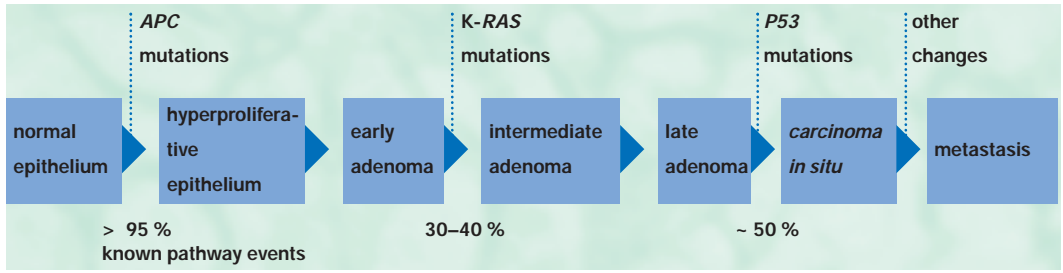


FIGURE 1: Sequential mutations of tumour genes and their probability of occurrence in the development of colorectal carcinoma (modified from [2]). Changes in the *APC* gene appear to be essential for the transition from normal to hyperproliferative epithelium. Changes in the *APC* pathway are demonstrable in al-

most all colorectal carcinomas. By contrast, only 30 to 40 % of tumours show point mutations in the *K-RAS* oncogene at the adenoma stage. Similarly, the tumour suppressor gene *P53* is mutated in only 50 % of tumours, whereas in the remaining 50 % other pathway events appear to play a role.

must be postulated as playing a role in the development of other tumours of this type.

Cellular processes can be affected at various points and by various genetic alterations

Individual cellular processes can be altered at various points and by various genetic lesions in different types of tumour. The development of retinoblastomas (RB) can serve as an example of this [3]. Retinoblastoma protein plays an important role in control of the cell cycle³. Central to regulation of the cell cycle is a class of proteins known as cyclins. These act not alone, but only in conjunction with cyclin-dependent kinases (CDK). These, in turn, influence other substances by phosphorylation⁴. Other cellular proteins such as p16^{INK4a} inhibit the activity of cyclin-dependent kinases.

In the G1 or resting phase of the cell cycle, RB protein binds transcription factors. In this way it prevents the cell from progressing from the G1 phase to the next phase, in which the DNA is copied. At the end of the G1 phase the cyclin D1-CDK4/6 complex phosphorylates RB protein, thereby detaching it from

3 The cell cycle is the series of phases of an actively dividing cell from one cell division to the next. It consists, in order, of the G1 phase, in which RNA and proteins are synthesised, the S phase, in which the DNA is replicated, the G2 phase, in which RNA and proteins are synthesised and the

spindle apparatus is formed, and the M (mitosis) phase, in which nuclear and cell division occur.

4 Attachment of an inorganic phosphate group (-PO₄) is known as phosphorylation.

Pathway	Alterations	Tumours
p16 ^{INK4a}	deletion, point mutation promoter methylation	glioma, carcinomas, sarcomas acute lymphatic leukemia familial malignant melanoma
↓		
cyclin D1	translocation amplification	mantle-cell-lymphoma, parathyroid adenoma, nasopharynx carcinoma, carcinomas of esophagus, urinary bladder, breast
↓		
CDK4	amplification	sarcomas, gliomas, familial, malignant, melanoma
↓		
RB	deletion, point mutation inactivation by E7 protein of papillomavirus	sarcoma, small cell lung cancer, carcinomas of urinary bladder, prostate and cervix, retinoblastoma

FIGURE 2: In human carcinomas, control of the cell cycle by the retinoblastoma (RB) pathway is disturbed by various genetic changes that lead to inactivation or loss of RB protein.

transcription factor E2F. Transcription factors regulate the activity of genes. In the case of E2F, the genes concerned regulate the cell cycle. As seen from Figure 2, the RB pathway can be influenced at various points in various types of tumour. All lesions ultimately result in inactivation of RB protein. The activity of a protein that plays a role at a particular point in a pathway can also be influenced by various genetic or epigenetic events. For example, the p16^{INK4a} protein can be inactivated by point mutations, the encoding gene can be lost, or the promoter region of the gene can be inactivated by methylation. The RB pathway is disturbed in most, though not all, malignant tumours in humans.

Combating cancer at the molecular level

Genetic diagnosis of a familial risk for cancer

People with germline mutations in tumour genes are at increased risk of developing cancer. According to the multistage model of carcinogenesis, tumour cells develop lesions in addition to the germline mutations that are present in all somatic cells. The predictive value of a mutation in a tumour gene depends on the likelihood of occurrence of additional pathway events. This is crucially important when therapeutic or invasive diagnostic measures are being considered. For example, members of MEN 2 (**m**ultiple **e**ndocrine **n**eoplasia type 2) families with a germline mutation in the *RET* proto-oncogene are highly likely to develop a medullary thyroid carcinoma [4]. This can be prevented by thyroidectomy in child-

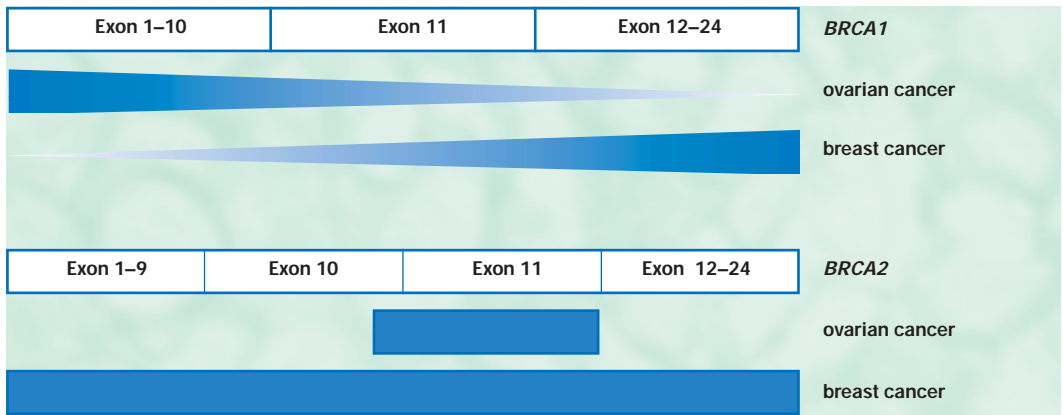


FIGURE 3: Correlation between the location of *BRCA1* and *BRCA2* gene mutations that interfere with protein biosynthesis and the occurrence of ovarian and breast cancer (figure taken with modifications from [6]).

hood. The predictive value of genetic defects in other tumour genes is considerably lower. In a recent study, the lifetime risk for breast cancer was assessed in Ashkenazi Jews carrying mutations in the breast cancer genes *BRCA1* and *BRCA2* but with no familial aggregation of breast cancer [5]. At the age of 70 years the penetrance of breast cancer was found to be less than 50 % in both *BRCA1* and *BRCA2* mutation carriers. The frequency of occurrence of malignant diseases can depend on the type and location of mutations in a given gene. For example, in carriers of mutations in the *BRCA1* and the *BRCA2* genes the probability that an ovarian or breast cancer will develop depends on the location of the mutations that interfere with translation and thus break the protein chain (Fig. 3) [6]. The probability of tumour development can depend on other genetic factors. In carriers of *BRCA2* mutations, for example, the risk for breast cancer is increased by the presence of a polymorphism in the *RAD51* gene [7]. Like the *BRCA* gene, the *RAD51* gene appears to be important for genomic stability. In carriers of *BRCA1* mutations, on the other hand, the presence of this polymorphism does not result in any increase in risk. Table 1 lists diagnostic and therapeutic measures that justify genetic diagnosis of predisposition to tumours [3].

Early diagnosis by demonstration of tumour genes

Though many problems are yet to be solved, demonstration of mutated tumour genes in body secretions and excretions is one

TABLE 2: Diagnostic and therapeutic measures that justify the diagnosis of a genetic predisposition to cancer.

Measure	Example
preventive diagnosis	colonoscopy in HNPCC and FAP patients
avoidance of invasive diagnostic measures	exclusion of cancer risk in members of HNPCC and FAP families
preventive therapeutic measures	thyroidectomy in patients from MEN2 families
screening of family members for germ line mutations cases of negative or unclear family history	patients with "spontaneous" tumours typical for hereditary cancer syndromes (e.g. medullary thyroid cancer)
	FAP familial adenomatous polyposis coli HNPCC hereditary non-polyposis colorectal cancer

of the most promising approaches to reducing cancer mortality. Carcinoma of the cervix is the only solid carcinoma for which a significant reduction in mortality has been shown to have occurred over the past few decades. This reduction has been achieved thanks to early diagnosis of cervical carcinoma by demonstration of cytological changes in screening examinations. The case of cervical carcinoma proves that early diagnosis of malignant tumours is possible if tumour cells are examined at a sufficiently early stage of the disease. Tumour cells can be shed in body secretions and excretions such as urine, pancreatic juice and feces. Where tumour cells are characterised by specific mutations or methylation patterns, sensitive and specific detection of such changes should permit early diagnosis of cancer. In the case of colorectal carcinoma, tumour cells are shed into the intestinal lumen at a very early stage (Fig. 4). Only later do tumour cells gain access to lymphatic and blood vessels, and only then do products of tumour cells find their way into the peripheral blood. At this relatively late stage the tumour is already well advanced. By contrast, demonstration of tumour genes in stool samples could permit diagnosis of neoplastic intestinal changes at a stage at which complete resection of the tumour is still possible.

What genetic changes can be used to help diagnose cancer?

When tumour genes are assessed in terms of their suitability for diagnostic purposes, a basic fact of tumour biology must be taken into account. It is becoming increasingly clear that the precise sequence of occurrence of genetic events during the clonal development of tumours is of the utmost importance. For example, mutations of the K-RAS gene are present relatively com-

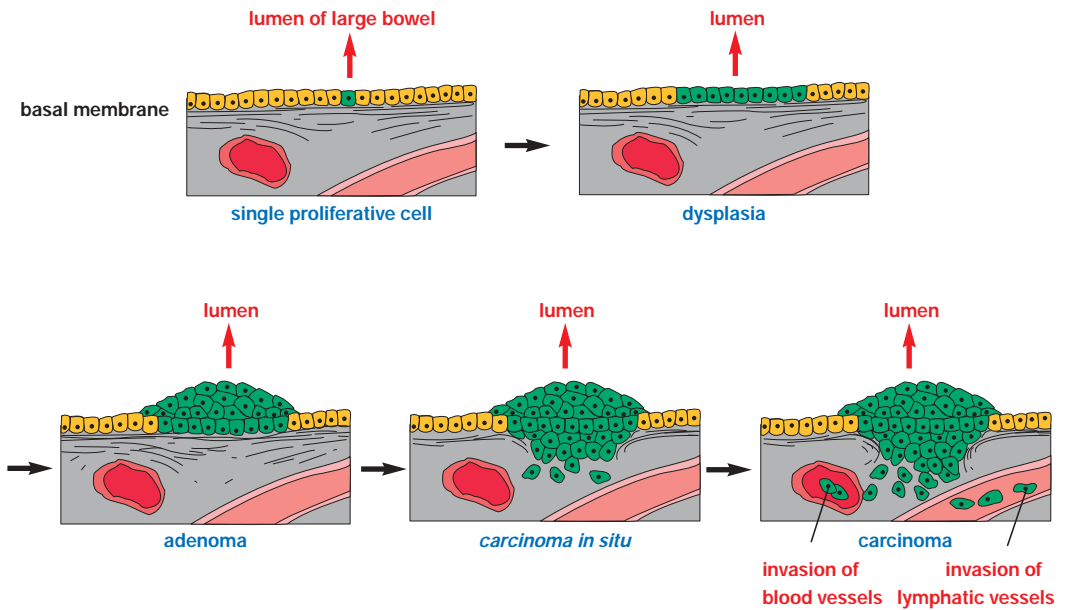


FIGURE 4: Schematic representation of the development of a colorectal carcinoma. Tumour cells are shed into the intestinal lumen from a very early stage. Only later do tumour cells gain access to lymphatic and blood vessels and thereby shed their products into

the blood. Demonstration of tumour genes in stool samples could permit detection of neoplastic bowel changes at a stage when complete, and therefore curative, excision of the tumour is still possible (figure taken with modifications from [14]).

monly in hyperproliferative large bowel lesions, but only rarely do these develop into colorectal carcinomas [8]. It appears that in the absence of *APC* mutations, *K-RAS* mutations induce apoptosis rather than proliferation. The fact that mutations in oncogenes are not necessarily tumour-specific is confirmed by the results of a recent study of ours on the identification of *K-RAS* mutations in samples of pancreatic juice taken from patients with pancreatic carcinoma or chronic pancreatitis [9]. *K-RAS* mutations were found in all samples taken from patients with pancreatic carcinoma, however, one mutation was also found in a sample taken from a patient with chronic pancreatitis who did not subsequently develop pancreatic carcinoma. The best target gene for early diagnosis of colorectal carcinoma would probably be *P53*, since mutations in this gene occur at a relatively late stage of colorectal carcinogenesis, namely at the *carcinoma in situ* stage.

Molecular approaches to therapy

Many molecular processes that promote the clonal development of tumours have been described.

Substances that counteract these processes have been developed. Processes occurring in tumours can be either inactivated or activated. In theory, it is more difficult to activate an inactivated pathway than to inactivate an activated pathway.

Activation of inactivated pathways

P53 is the only tumour gene that is very commonly inactivated in human tumours. There are a number of ways in which inactivation of *P53* can potentially be reversed [10]. Nevertheless, methods of reactivating *P53* and other pathways that are inactivated in tumours are still far from reaching the stage of clinical trials. On the other hand, early clinical experience is now available on substances that inactivate activated pathways.

Inactivation of activated pathways

Though this approach is conceptually more straightforward, relatively few substances have so far proved to be effective in clinical trials. The first such substance, all-*trans*-retinoic acid, was used to treat promyelocytic leukemia (PML) even before the molecular defect that it corrects had been identified. This substance acts on a tumour-associated fusion protein derived from retinoic acid receptor- and the PML protein. A second such substance is trastuzumab (proprietary name: Herceptin®), a monoclonal antibody that inhibits the actions of the protein encoded by the oncogene *ERBB2* (also known as HER2/neu) in a proportion of patients with breast cancer. Neither of these drugs, however, induces sustained remission.

Inhibition of ABL kinase in leukemia

STI571 is a newly developed substance that is used to treat chronic myeloid leukemia (CML) and acute lymphatic leukemia (ALL). In over 95 % of patients with CML and in a proportion of patients with ALL, reciprocal translocations between chromosomes 9 and 22 lead to the formation of hybrid genes that encode fusion proteins containing ABL⁵ kinase and an incomplete BCR⁶ protein. STI571 inhibits the kinase activity of the BCR-ABL fusion proteins by competing for binding to the ATP binding site. As it also binds to normal ABL kinase, it is not specific for the oncogenic variant of the enzyme.

The genetic instability of tumour cell clones leads to the development of resistance

In an initial study, 53 out of 54 patients in the early chronic phase of the disease who were treated with STI571 went into sustained remission with few side effects [11]. A proportion of patients in blast crisis likewise responded initially to the substance, however, all the ALL patients, and a high proportion of the CML patients, in blast crisis subsequently relapsed [12]. At the molecular level, resistance to STI571 was brought about by two different mechanisms [13]. In a proportion of the leukemia cells, resistance was associated with ABL gene amplification. The second mechanism shows how the genetic instability of tumour cells makes it difficult for drugs to exert a lasting effect. In the STI571 binding pocket, the amino acid threonine was replaced at position 315 by isoleucine. This amino acid substitution prevents binding of the drug, whereas kinase activity is retained. It is notable that the same amino acid substitution occurred in all six patients with a point mutation. It is conjectured that only a few amino acid substitutions, or possibly only this one, inhibit binding of STI571 (see Fig. 5).

It appears that by activating the corresponding signal pathway, the BCR-ABL fusion protein plays an essential role in malignant transformation in CML. This assumption is supported by the fact that the gene that encodes the fusion protein is either mutated or amplified in all patients who are resistant to STI571. In other types of tumour, pathways can – as described above – be disturbed at various points or substituted with a different pathway. The use of DNA arrays makes it possible to distinguish between pathways in tumours by analysing mRNA expression profiles. These are extremely heterogeneous in carcinomas. In breast cancer, for example, gene expression profiles have been found to vary enormously between individuals. This variability is multidimensional, i.e. many different gene groups show mostly independent patterns of variation. From this it follows that different processes are activated or inactivated in different tumours. The fact that the expression profiles of primary tumours and their metastases resemble each other more than do those of tumours from different patients suggests that individual pathways are retained during the progression of cancer in a given patient.

5 ABL refers to a cellular counterpart of the oncogene of the **Ab**elson leukemia virus.

6 BCR stands for **breakpoint cluster region**.

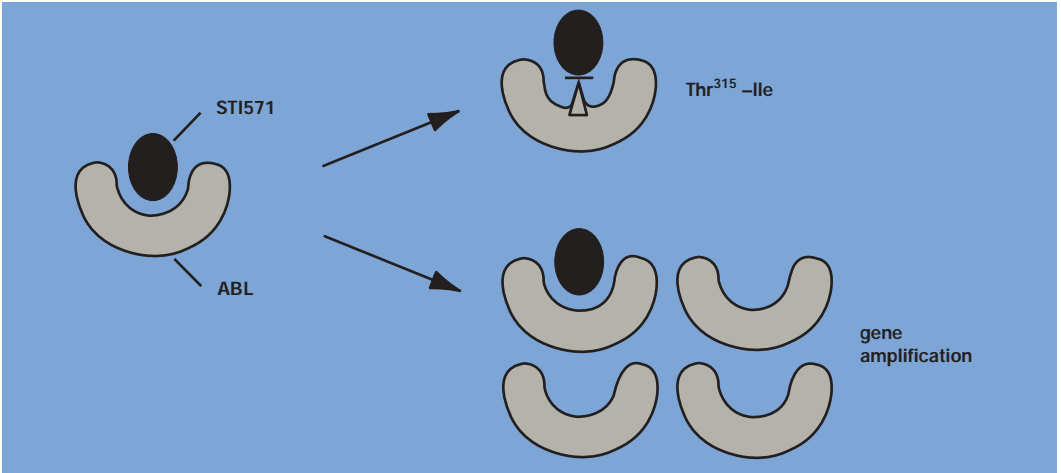


FIGURE 5: Where a protein plays an important role in a neoplastic process, point mutations or amplification of the gene that encodes that protein could result in resistance to drugs that interact with the protein concerned.

Outlook: fight not (just) tumour cells, but structures that supply them

Genomic heterogeneity and instability are major obstacles to molecular tumour therapy. The problems are basically similar to those that arise in the treatment of HIV infection. Both HIV and tumour cells are characterised by a high mutation rate, however, the number of genes, and thus the number of possible variants, is incomparably higher in tumour cells than in HIV. Given the problem of the genomic heterogeneity and instability of tumour cells, a more promising therapeutic target might be the supply structures of solid tumours. The most important of these are the blood vessels that supply the tumour with oxygen and nutrients. Solid tumours can exceed a diameter of about 1 mm only if they are supplied by blood vessels. The endothelial cells that are involved in the formation of blood vessels are genetically stable and are therefore suitable targets for molecular therapy. It has been shown in various animal models that tumour growth can be inhibited by interfering with angiogenesis (the development of blood vessels) in a tumour. Since, with few exceptions (e.g. pregnancy, maturation of the corpus luteum), endothelial cells have a low rate of division in adults, antiangiogenic therapy should not have any severe side effects. Results obtained recently in clinical trials suggest that tumour growth can be inhibited not just in experimental models, but also in cancer patients, by means of antiangiogenic therapy.

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