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Molecular genetics and the assessment of human cancers

Paul D.P. Pharoah and Carlos Caldas

The past 20 years have seen rapid advances in the understanding of the biology of human cancers, and a large body of evidence now supports the idea that accumulated genetic changes underlie the development of neoplasia. In this article, we have reviewed the current research into the genetic bases of cancer and discussed the potential clinical applications of recent advances, with particular reference to the possibility of using molecular genetic tests for pre-symptomatic screening, clinical diagnosis and clinical staging. The possibility that the genetic profile of a tumour, its 'molecular fingerprint', will improve the ability of oncologists to predict tumour behaviour and thus help to determine optimum treatment has also been considered. Although the potential for the application of molecular genetic technologies is enormous, these technologies have yet to be subjected to rigorous evaluation in a clinical setting, and much work needs to be done before they are adopted for use in routine clinical care.

Worldwide, 10 million new cases of cancer will be diagnosed during the year 2000 (Ref. 1); unfortunately, the overall survival rate for such patients has changed little during the past 20 years, despite rapid advances in the understanding of the biology of human cancer. However, the elucidation of many of the genetic events that underlie the cancer process, combined with technical developments that have made it possible to work directly with tumour specimens and clinical samples, forms the basis of novel diagnostic and prognostic approaches that might help to improve the survival rate of such patients.

Molecular profiling, in its widest sense, unravels the molecular processes taking place inside a cell by detecting abnormalities in its genes, proteins and protein networks. In this article, we have discussed some of the recent

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advances in molecular genetics that have made it possible to identify single cancer cells among a population of cells (e.g. a tissue sample), and to classify populations of cancer cells according to their molecular genetic profile. The potential for using DNA-based methods in screening, diagnosis and staging of cancer has been explored, and the possible clinical implications of molecular profiling outlined. This review excludes RNA and protein-based studies, which have recently been reviewed elsewhere (Ref. 2, and references therein).

The genetic bases of cancer

One of the most important discoveries in cancer biology during the past two decades has been that cancers arise as a result of cumulative genetic changes in somatic cells (Fig. 1). The progression of a tumour from normal cells to pre-cancerous ones, to cancer and then on to local invasion and finally metastasis is the result of the clonal expansion of cells that have acquired a selective growth advantage, which allows them to outnumber neighbouring cells. This advantage is the result of changes in genes that control cellular proliferation and cell death (Ref. 3). These changes involve alterations or mutations in specific oncogenes, and are generally divided into two major categories: those that activate proto-oncogenes (e.g. *K-ras* and *H-ras*), which promote cellular proliferation or inhibit cell death; or those that inactivate tumour suppressor genes [e.g. *APC* and *P53* (also known as *TP53*)], which inhibit cell proliferation or promote cell death. Although a normal cell is thought to require five or more mutations to become cancerous (Ref. 3), the probability of this happening is increased because the genetic material in pre-cancerous and cancer cells is intrinsically unstable, a characteristic known as genetic instability (Ref. 3; see below).

The accumulation of genetic changes in cells during cancer development and progression has most comprehensively been described for colorectal cancer (Ref. 4). In this cancer, when the tumour suppressor gene *APC* is inactivated, a normal mucosal cell in the colonic epithelium can proliferate abnormally and become a small adenomatous polyp. Mutations in oncogenes, such as *K-ras*, or in tumour suppressor genes, such as *P53* or *DCC*, occur subsequently; these mutations can lead to the transformation of the polyp into a larger adenoma, from which a carcinoma can subsequently arise (Fig. 1). Mismatch-repair genes recognise and correct the mismatches that occur during the replication of DNA. In tumours that have inactivation of the mismatch-repair genes (15%), whether sporadic or in the context of hereditary nonpolyposis colorectal cancers (HNPCC), the rate at which these mutations occur is accelerated by the genetic instability. *APC* has been described as the 'gatekeeper' of the colonic epithelium, and in colorectal cancer, there is good evidence that disturbance of the *APC* pathway is required for the initiation of the neoplastic process. However, for most other human tumours, such detailed knowledge of early genetic events is lacking. This lack of knowledge is critical for the development of clinical applications, because knowledge of these early events is a prerequisite of any successful early diagnosis or screening strategy.

Although less-well understood, epigenetic changes, which are defined as heritable alterations in gene function that are mediated by factors other than changes in the primary DNA sequence, might also be important in tumorigenesis. The methylation of DNA in the gene promoter region is one such change that can alter gene expression. Actively transcribed genes tend to have low levels of DNA methylation at their promoter regions, whereas transcriptionally silent genes are often heavily methylated at their 5' end (Ref. 5). DNA methylation could, therefore, potentially be a non-mutational mechanism that disrupts the function of tumour suppressor genes or increases the expression of proto-oncogenes. Another epigenetic change that might alter gene expression is genomic imprinting, a reversible modification of DNA that causes differential expression of maternally and paternally inherited homologous genes (Ref. 6). Loss of imprinting (LOI) is a recently discovered alteration that is associated with cancer, which involves the expression of genes that are usually not expressed in a manner that is specific for the parent of origin; a particular gene is expressed only from one of the two alleles, depending on which parent it was inherited from. Loss of imprinting might include activation of the normally silent copy of growth-promoting genes, or silencing of the normally transcribed copy of tumour suppressor genes.

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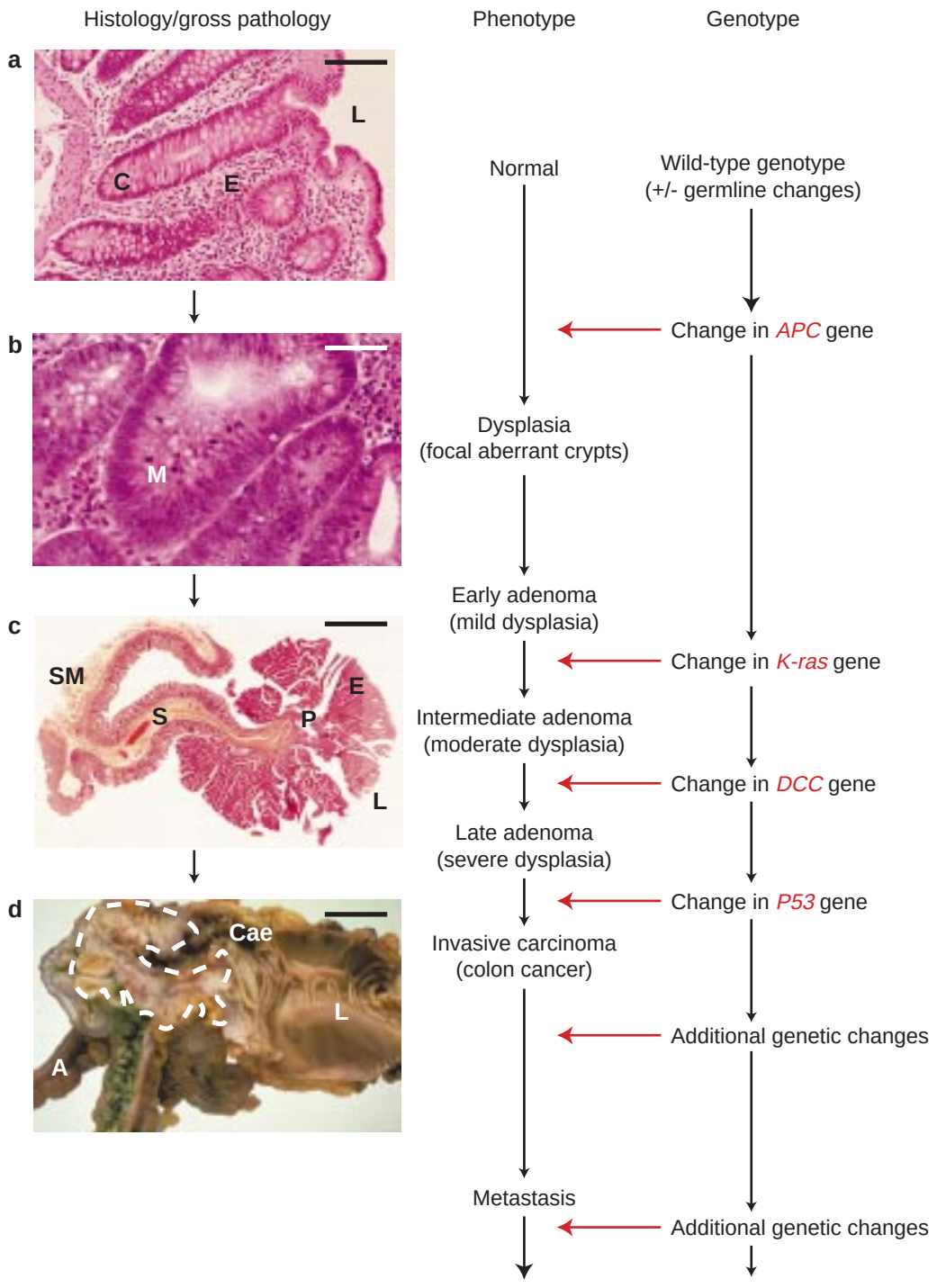

Sequential genetic changes that are associated with dysplasia and malignant neoplasia of the colon

Figure 1. Sequential genetic changes that are associated with dysplasia and malignant neoplasia of the colon *(see next page for legend)* **(fig001ccc).**

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Figure 1. Sequential genetic changes that are associated with dysplasia and malignant neoplasia of the colon. (a) Light micrograph of a section (stained with Haematoxylin and Eosin) of normal colonic epithelium (E), showing simple, tubular colonic crypts (C), lined by absorptive epithelial cells with basal nuclei interspersed with occasional mucus-secreting goblet cells. L = the lumen of the gut. Scale bar ≈ 180 μm. (b) Light micrograph [at a higher magnification than in (a)] of a section (stained with Haematoxylin and Eosin) of moderately dysplastic colonic epithelium. The gland architecture is distorted with loss of the normal linear array of the nuclei of colonic epithelial cells; mitotic figures (M) are seen moderately frequently, away from the basal layers. Scale bar ≈ 90 μm. (c) Photograph of exophytic growth (stained with Haematoxylin and Eosin) of a polyp (P; or adenoma) of the rectum. The architecture of the tissue is distorted, and the glandular epithelium (E) shows the cytological features of moderate dysplasia; there is no invasion into the underlying lamina propria. S = stalk of the polyp; SM = submucosa. Scale bar ≈ 3.8 mm. (d) Photograph of a pathological specimen (fixed with formalin) of the caecum (Cae) and appendix (A), showing an infiltrating carcinoma. The tumour (outlined with a dashed, white line) has spread into the wall of the bowel, which has undergone gross thickening to more than 1 cm. Scale bar ≈ 6 cm. These phenotypic changes (left and centre panels) are typical of the progression of neoplastic lesions of the colon and are associated with a sequence of molecular genetic changes (right-hand panel), which have been studied in some detail for this cancer. These changes may be either somatic (affecting only the tumour cells) or germline (affecting all the cells in the body). The initial change is mutation in both copies of the adenomatosis polyposis coli (*APC*) gene. In familial APC, one copy of the *APC* gene is mutated in the germline, so predisposing the individual to colon cancer. This is followed by changes in the proto-oncogene *K-ras*, the gene deleted in colorectal cancer (*DCC*) and the *P53* gene (also known as *TP53*). Other genes are also changed, particularly in later stages and can affect the ability of the tumour cells to spread and metastasise **(fig001ccc).**

The detection either of gene mutations or of genetic instability in populations of cells might be a useful clinical tool in the diagnosis of cancer because they are both biological characteristics of tumour progression. In addition, specific genetic changes can be predictive of tumour behaviour (e.g. response to treatment); they can, therefore, define prognosis and might help to optimise treatment.

Detection of mutations in cancer cells

A variety of mutations can alter the function of individual genes; these include insertions, deletions, duplications and inversions of genes, and substitutions of single base pairs. Within the coding sequence, insertions, deletions, duplications and inversions usually result in truncation (shortening) of the gene product, whereas single-base changes can either alter the amino acid sequence of the full-length gene product or result in truncation. Mutations outside the coding sequence can affect transcription, translation and mRNA (messenger RNA) splicing and processing. A gene can be tested for the presence of a mutation after it has been amplified using the polymerase chain reaction (PCR), which allows the analysis of very small quantities of tissue; a moderately sensitive PCR-based assay can detect one abnormal cell in 1 x 10^5 cells. However, such a test requires knowledge not only of the nucleotide sequence of the gene, but also of the specific alteration in the gene. In human cancer, many different mutations can affect the function of a gene, such that testing a gene for all possible or known mutations can be time-consuming and therefore expensive. This fact limits the potential use of gene mutations as molecular markers in clinical testing; however, the study of mutations remains very important for clinical research.

Detection of genetic instability in cancer cells

Microsatellite instability

The detection of genetic instability, which is an intrinsic property of cancer cells, is more straightforward using PCR-based technology than testing for mutations (Fig. 2). Genetic instability frequently results in subtle sequence alterations within microsatellites, which are simple tandem repeats of DNA nucleotides that occur abundantly and randomly throughout the human genome. In hereditary nonpolyposis colorectal cancers, microsatellite instability is due to a defect in the ability of cells to repair nucleotide mismatches during DNA replication because of mutations in mismatch-repair genes (Ref. 4). However, the mismatch-repair genes that have been cloned, to date, do not appear to be mutated in the majority of colonic cancers studied or in cancers arising at other sites (Ref. 7). A milder form of microsatellite instability, usually involving larger repeats and sparing dinucleotide repeats, has been demonstrated in most types of human solid tumours; thus, it has been suggested that

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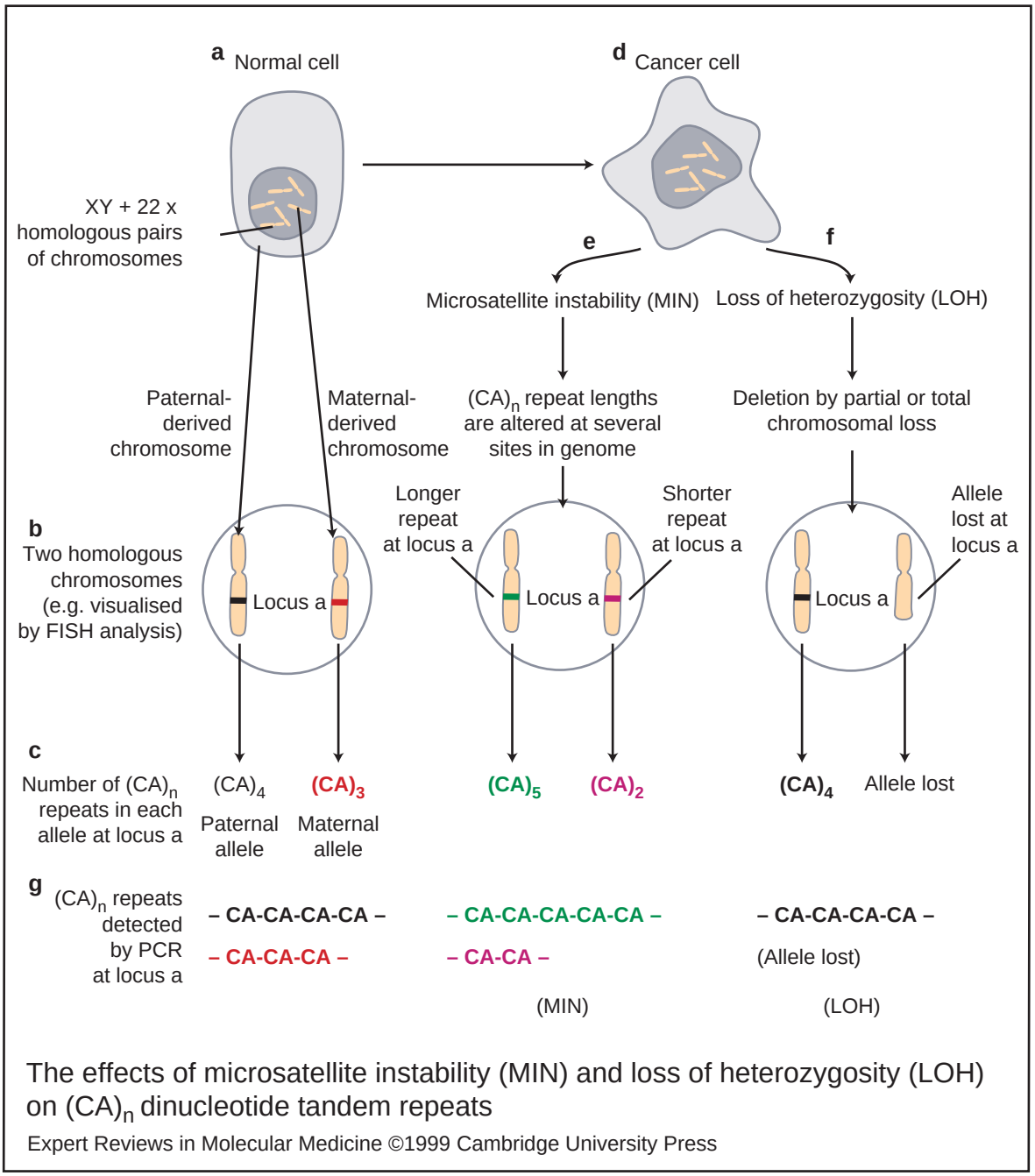

The effects of microsatellite instability (MIN) and loss of heterozygosity (LOH) on $(CA)_n$ dinucleotide tandem repeats

Figure 2. The effects of microsatellite instability (MIN) and loss of heterozygosity (LOH) on $(CA)_n$ dinucleotide tandem repeats *(see next page for legend)* **(fig002ccc).**

microsatellite alterations might be useful as markers to detect cancerous cells in pathological samples (Ref. 8).

Chromosomal instability

Genetic instability can also manifest as gross chromosomal changes, which are probably due to defects in mitotic segregation or recombination during cell division (Ref. 9). Such changes can often be detected using relatively simple and reproducible techniques. Fluorescent in-situ hybridisation (FISH) can be used to identify gross chromosome alterations such as an excess or loss of one or more chromosomes (aneuploidy) and breakage of two chromosomes, with transfer and fusion of parts of the broken fragments onto each

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Figure 2. The effects of microsatellite instability (MIN) and loss of heterozygosity (LOH) on $(CA)_n$ dinucleotide tandem repeats. (a) Normal human cells have 22 pairs of 'matched' (homologous) chromosomes and a pair of sex chromosomes (either XX or XY). Each one of a pair of chromosomes is inherited from either the mother (maternal) or father (paternal), and because of normal polymorphism (or accumulated 'silent' mutations) a given position (locus) on the chromosome pair will have a (slightly) different sequence (they are, therefore, heterozygous). (b) These differences cannot currently be visualised using fluorescently labelled specific probes and fluorescent in-situ hybridisation (FISH). (c) Human cells have many randomly arranged microsatellites throughout their genome. Microsatellites are simple tandem repeats of DNA nucleotides [e.g. $(CA)_n$] of unknown function, which are of great use to geneticists because they can be used as markers of small changes in the genome. For example, a normal human cell might have three CA repeats on one allele and four repeats on the other. (d) Cells that eventually become transformed and can form a tumour (cancer) can have single or multiple mutations in their genes (or in the sequences that control the genes). A more generalised instability can also affect their genome, resulting in either: (e) microsatellite instability (MIN) or (f) loss of heterozygosity (LOH), or both. For example, MIN can be due to a defect in the ability of cells to repair nucleotide mismatches during DNA replication, and LOH can result from the loss of larger pieces of the chromosome. Changes in the number of repeats can be detected using the polymerase chain reaction (PCR), which uses specific primers to amplify the DNA in a sample of tumour tissue and compares it with the normal tissue **(fig002ccc).**

other (translocation). Structural alterations such as the loss of a piece of a chromosome (deletion) or amplification of part of a chromosome can be identified by techniques such as comparative genomic hybridisation.

PCR-based methods have also been used to demonstrate gene loss in cancer. This approach relies on an individual being heterozygous at a specific genetic locus, such as at a microsatellite, where the number of tandem repeats is often polymorphic. A heterozygous individual will have one number of repeats at that locus on one chromosome, and a different number at the same locus on the other chromosome (Fig. 2). Tumour tissue from a cancer patient who is heterozygous at a particular locus (termed an informative individual) can be analysed using PCR, and compared with normal tissue that has been analysed in the same way. If one of the alleles that is present in the normal DNA of the heterozygous individual is missing from the DNA of the tumour cell, the tumour is said to have undergone a reduction to homozygosity or a loss of heterozygosity (LOH; see Fig. 3).

Many studies have investigated the prevalence of genetic abnormalities in various cancers, and Table 1 summarises the prevalence of microsatellite instability and of mutations in the oncogene *K-ras* and the tumour suppressor genes *P53*, *APC*, *DPC4* and *P16* in four common human cancers.

Although studies of gene expression in cancer are outside the scope of this article, a new technique called serial analysis of gene expression (SAGE) is likely to produce substantial insights into the cellular biology of cancer, and will help to identify novel molecular markers of the neoplastic process. SAGE is one of several new techniques for comprehensive transcript expression analysis. (Further details of these can be obtained from the Cancer Genome Anatomy Project website at: http://www.ncbi.nlm.nih.gov/ncicgap/). Powerful technologies such as SAGE can now be used systematically to 'mine' the genome for tumour markers; the potential of this technology has been realised recently by scientists from Johns Hopkins University (Baltimore, MD, USA). Zhang and colleagues have used SAGE to identify 45 000 genes that are expressed in human pancreatic cancer cells, and found that 183 of these genes were expressed at elevated levels, compared with those of normal pancreatic cells (Ref. 10). They went on to show that secreted protein encoded by one of the overexpressed genes (*TIMP-1*, which encodes tissue inhibitor of metalloproteinase 1) would be a useful serum marker for human pancreatic cancer (Ref. 11).

Molecular diagnosis and screening for cancer

The possibility of identifying neoplastic cells in clinical samples using molecular genetic techniques was first demonstrated in 1991, when Sidransky and colleagues detected cells in urine samples from patients with bladder cancer that had mutations of the *P53* tumour suppressor gene, which were identical to the mutations found in the cells of the primary tumour (Ref. 12). The feasibility of identifying neoplastic cells in different clinical samples (some of which can be obtained non-invasively) from a variety of different tumours and using a variety of

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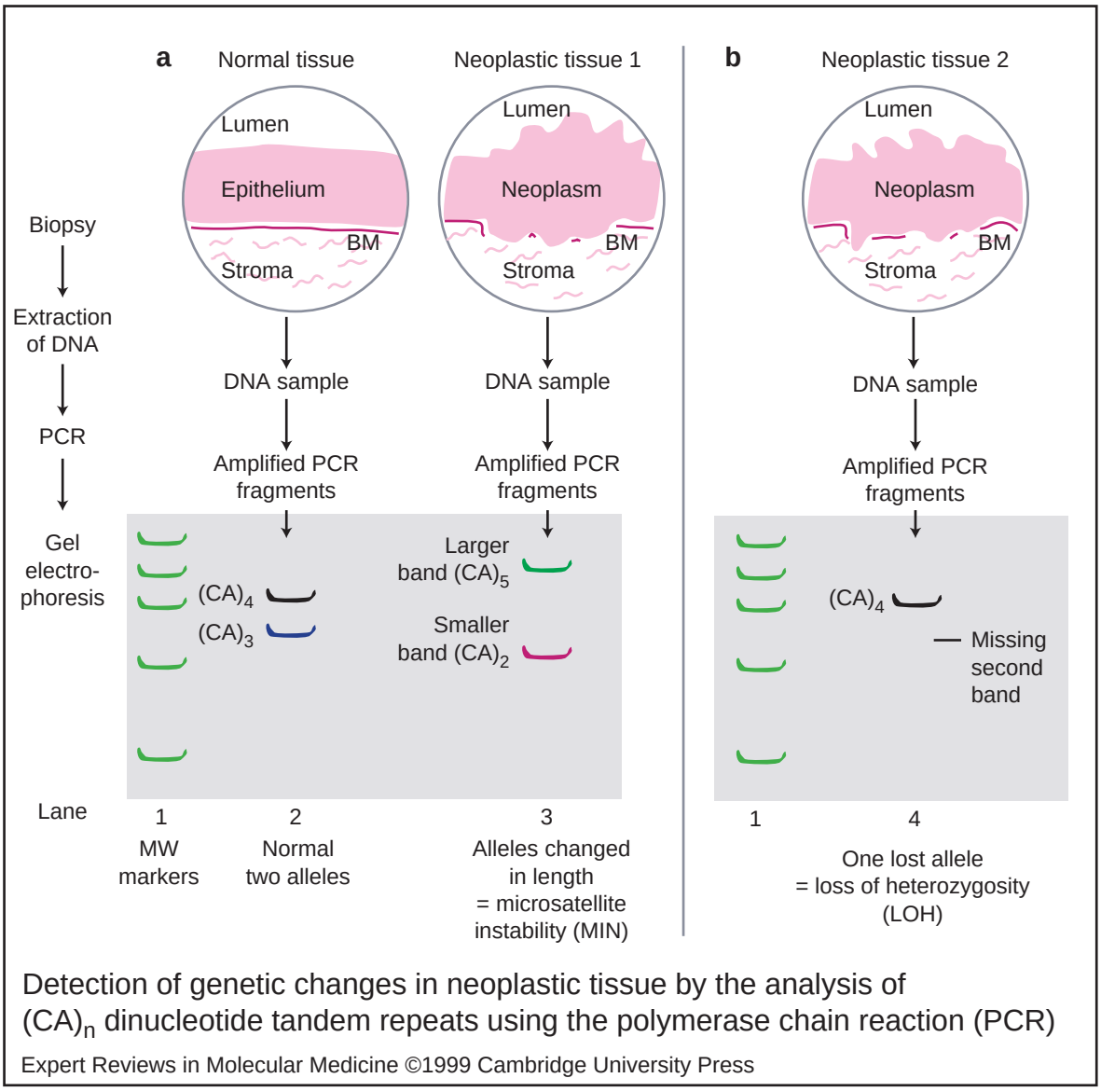

Figure 3. Detection of genetic changes in neoplastic tissue by the analysis of $(CA)_n$ dinucleotide tandem repeats using the polymerase chain reaction (PCR). (a) By histological analysis of a clinical sample (biopsy), cancerous tissue can be distinguished from surrounding normal epithelium. Microdissection can be used to separate cancer cells and normal epithelial cells, and to extract DNA from each. To find out more about genetic changes in the cells, which are often associated with cancer, this tissue is compared with normal tissue (or blood cells) from the same individual. DNA is extracted from each tissue and a set of polymerase chain reaction (PCR) primers is used to amplify the DNA and analyse the size of the resulting fragments [by comparing them with standard molecular weight (MW) markers] using gel electrophoresis. In this example, the neoplastic tissue is found to have two bands with different lengths of tandem dinucleotide repeats [microsatellites $(CA)_5$ and $(CA)_2$], compared to the normal tissue, which is typical of microsatellite instability (MIN). (b) A similar procedure is performed on another sample of neoplastic tissue and only one band of normal repeat length is amplified, which is typical of loss of heterozygosity (LOH) **(fig003ccc).**

different molecular genetic markers has since been demonstrated in many studies, which are summarised in Table 2. Other clinical samples that could be used for molecular diagnosis or screening include fine-needle aspirates (from any site), nipple aspirates to screen for breast cancer,

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Table 1. Prevalence (%) of some common somatic genetic alterations in common sporadic cancers[a] (tab001ccc)

Cancer	Genetic alteration					
	K-ras mutation[b]	*P53* mutation[c]	Microsatellite instability[d]	*APC*[c]	*DPC4*[c]	*P16*[c]
Breast	Unknown	20–70 (Ref. 73)	0–29 (Ref. 7)	–	–	–
Colorectal	40–50 (Ref. 16)	50–75 (Ref. 17)	10–29 (Ref. 7)	60–80 (Refs 74, 75)	–	–
Lung	30 (Ref. 76)	50–80 (Ref. 76)	0–49 (Ref. 7)	–	–	–
Pancreas	70–85 (Ref. 77)	50–70 (Ref. 77)	<5 (Ref. 78)	–	50 (Ref. 79)	80 (Ref. 80)

[a] The table shows a summary of the prevalence (%) of specific alterations in sporadic cancers, as published in several studies.
[b] *K-ras* is a proto-oncogene that is activated in human cancers by point mutations.
[c] *P53*, *APC*, *DPC4* [also known as mothers against decapentaplegic, *Drosophila* homologue 4 (*MADH4*)] and *P16* are all tumour suppressor genes, which are located at chromosomes 17p, 5q, 18q and 9p, respectively.
[d] Microsatellites are short tandem repeats of nucleotides, which are dispersed throughout the genome.

and cerebrospinal fluid to screen for cerebral neoplasms.

Molecular genetics in screening and diagnosis

Clinical screening is the testing of apparently asymptomatic individuals to classify them into those who are likely to have disease and those who are not; that is, the detection of disease in its pre-clinical phase. The possibility of using molecular markers to detect cancer in the pre-clinical phase has been demonstrated by several case reports: one of these showed that a *P53* mutation could be detected in a urine sample nine years before the diagnosis of bladder cancer was made (Ref. 13). Similarly, a mutation in *K-ras* has been detected (1) in pancreatic 'juice' that was collected from a patient three years before pancreatic adenocarcinoma was diagnosed (Ref. 14), and (2) in colonic washings of patients who were at high risk of developing colorectal cancer. In addition, oncogene mutations have been found in the sputum of patients before the diagnosis of lung cancer (Ref. 15). However, in clinical practice in the future, the usefulness of particular molecular methods will depend on several factors including: (1) the sensitivity, (2) the specificity, (3) the positive-predictive value of the tests, (4) the feasibility and cost of carrying out the tests in large numbers, and (5) the acceptability of the test to patients.

Sensitivity of clinical tests

The sensitivity of a clinical test for cancer is defined as the proportion of the total number of patients with cancer tested that is correctly identified by the test. For a molecular genetic assay, this will depend on two factors: (1) the proportion of cancer cells in which the abnormality is found, and (2) the ability of the test to detect abnormal cells in a clinical sample. We know that mutations, deletions, translocations

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Table 2. Studies that have used molecular genetic alterations to identify tumour cells in clinical samples[a] (tab002ccc)

Cancer	Type of clinical sample	Gene in which mutation(s)/ alteration(s) were detected	Refs
Bladder	Urine	*P53*[b]	12
		Microsatellite instability[c] and loss of heterozygosity	19
Colorectal	Stool	*K-ras*[d]	81, 82, 83, 84
	Whole-gut lavage fluid	*K-ras*, *P53*	18
	Serum	K-ras, *P53*	26, 27, 28, 29
	Endoscopic biopsy specimens	*K-ras*	85
	Colonic washing	*K-ras*	86
Head and neck	Serum	Microsatellite instability and loss of heterozygosity	31
	Saliva	Microsatellite instability and loss of heterozygosity	87
Oral/pharyngeal	Saliva	*P53*	88
Lung	Sputum	*K-ras*	15
	Plasma	Microsatellite instability	30
	Broncho-alveolar lavage fluid	*K-ras*	89
Pancreas	Plasma	*K-ras*	32
	Duodenal fluid	*K-ras*	90
	Fine-needle aspirate	*K-ras*	91, 92, 93, 94
		P53	92
	Stool	*K-ras*	20
	Peritoneal effusion	*K-ras*	95
	Pancreatic juice	*K-ras*	96, 97, 98, 99
	Cytology smears	*K-ras*	100
	Bile	*K-ras*	101

[a] This table shows a summary of the findings of a large number of studies, in which gene mutations or other genetic alterations were found in clinical samples. [b] *P53* is a tumour suppressor gene, which is located at chromosome 17p. [c] Microsatellites are short tandem repeats of nucleotides, which are dispersed throughout the genome. [d] *K-ras* is a proto-oncogene that is activated in human cancers by point mutations.

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and duplications of many genes can contribute to tumour formation, depending on the tissue of origin; however, no one genetic change is common to all cancers, a fact that will limit the sensitivity of any test to detect a cancer. For example, mutations in *K-ras* are associated with only 40–50% of colorectal cancers (Ref. 16), whereas *P53* mutations occur in 50–75% of cases (Ref. 17). The successful detection of abnormal cells in clinical specimens requires the use of methods that selectively enrich for specific mutations. Thus, genetic detection is currently limited to the study of point mutations at a few known, fixed sites. Successful genetic analysis of the four most common mutations in *K-ras* and of *P53* mutations would potentially detect only 60% of colorectal cancers (Ref. 18). Microsatellite instability might prove to be a more useful screening test for some tumours; for example, microsatellite analysis has been found to have a sensitivity of 95% in detecting bladder cancers that had been confirmed by cystoscopy (endoscopic examination of the interior of the bladder), compared with only 50% sensitivity for conventional cytology (Ref. 19).

Specificity of clinical tests

The specificity of a clinical test for cancer is defined as the proportion of the total number of people tested that is correctly identified by the test as not having cancer. Because some specific genetic abnormalities are thought to be a characteristic of cancer cells, some molecular genetic tests should be highly specific. However, other genetic abnormalities can also be characteristic of dysplastic and pre-cancerous cells; thus, until more is known about the natural history of such lesions (for example the proportion of lesions that progresses to invasive cancer, and over what time scale), the specificity of such markers to cancer is hard to predict. For example, mutations in *K-ras* have been found in patients who have chronic pancreatitis with ductal hyperplasia (Refs 20, 21, 22); whether this change represents a very high probability of progression to pancreatic adenocarcinoma is not known. Mutations in *K-ras* have also been found in cells in aberrant colonic crypts, and *P53* mutations in normal skin cells.

The positive-predictive value of tests

The positive-predictive value of a clinical test is defined as the proportion of the total number of people tested that tests positive and really has the disease. Thus, it is a function of both the sensitivity and specificity of the test and of the prevalence of the 'condition' in the population that is being screened. Because the prevalence of cancer in screened populations is usually low, sensitivity and specificity must be very high if the positive-predictive value of a cancer screening test is to be acceptable for clinical use.

Molecular staging of cancer

One of the cornerstones in the clinical management of cancer patients is the accurate determination of the extent of local, regional and distant spread of a cancer (termed clinical staging), because this provides prognostic information and also helps to determine treatment. Currently, assessment is based on the histopathological study both of cells found at the margins of tissue removed during surgical resection of cancer, and of cells found while draining lymph nodes of the cancerous area. The problem with this approach is that small foci of metastatic cancer can be missed, either because the sampling is insufficient or because the analysis of the cell morphology is uninformative. The ability to use molecular markers to detect a small number of cancer cells in pathological specimens should improve clinical staging.

Molecular-based staging has been evaluated in a recent study of 25 patients with squamous cell carcinoma of the head and neck who, on the basis of a negative histopathological assessment, had had complete tumour resection (Ref. 23). In over half of the patients, *P53* mutations were found using molecular analysis in the surgical resection margin. In addition, the molecular analysis identified the presence of neoplastic cells in one quarter of the lymph nodes that were initially considered to be 'negative' by histopathological assessment. Furthermore, five out of 13 patients whose tumour margins were assessed to be 'positive' (using molecular staging) developed local recurrence of the cancer, compared with none of the 12 patients whose margins were assessed to be negative using molecular staging.

In colorectal cancer, mutations of *K-ras* and *P53* have been used to identify the presence of cancer cells in lymph nodes that had no histological evidence of metastasis at the time of surgery (Ref. 24). Tumour recurrence occurred in 27 out of 37 patients with lymph nodes that had mutations in either *K-ras* or *P53*, compared with none of the 34

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patients who were assessed as negative using these tests for lymph node metastasis (Ref. 25).

Molecular techniques have been used successfully to identify the presence of cancer cells in the systemic circulation in patients with colorectal cancer (Refs 26, 27, 28, 29), small-cell lung cancers (Ref. 30), head and neck cancers (Ref. 31), and pancreatic cancer (Ref. 32). Although the presence of circulating tumour cells (identified by mutations in *K-ras*) was found to be a sensitive marker for subsequent relapse in one of these studies (Ref. 26), it remains to be seen whether the detection of cancer cells within the systemic circulation usually correlates with either disease progression or prognosis.

Fluorescent in-situ hybridisation

The potential clinical value of chromosome analysis using fluorescent in-situ hybridisation (FISH) has been demonstrated by reports of the detection of single metastatic cells in lymph node tissue from a patient with renal cell carcinoma (Ref. 33), and the detection of a gene deletion in single metastatic tumour cells in the excision margin of a primary cutaneous melanoma (Ref. 34).

Molecular fingerprinting

Because molecular genetic changes in general are responsible for the biological characteristics of neoplastic cells, it is likely that specific genetic alterations will predict the behaviour of such cells. It is hoped that the time will come when oncologists will be able to characterise the molecular fingerprint of a tumour, which represents the genetic profile of a tumour. Such a fingerprint might then provide clues to help to determine whether a tumour will be fast or slow growing, whether it will metastasise, and whether it will respond to particular treatment regimens. Although the molecular fingerprinting of tumours is not yet a clinical reality, the value of molecular markers in clinical practice has already been suggested by many research studies of the clinical implications of various genetic markers for different types of tumours.

The molecular fingerprint and prognosis

Breast cancer

The first report of the prognostic significance of a molecular marker was in a study of human breast cancer, when it was reported that amplification of the *HER-2/neu* receptor oncogene was associated with earlier than average relapse and reduced overall survival (Ref. 35). This raised the possibility that the status of this gene might predict the response to treatment, and two large studies examining the role of elevated levels of the HER-2/Neu protein in predicting the response to treatment have recently been published. The first of these compared clinical outcome in patients with breast cancer who were lymph-node positive and randomised to receive low-, medium- or high-dose adjuvant chemotherapy, and found that improved survival with high-dose doxorubicin-based chemotherapy was limited to patients with tumours that had elevated levels of HER-2/Neu protein (Ref. 36). The second compared survival in patients with breast cancer who were lymph-node positive, but hormone-receptor negative and randomised to receive adjuvant chemotherapy with or without doxorubicin. Treatment with doxorubicin was found to confer survival benefit, but this was also confined to patients with tumours that had elevated levels of HER-2/Neu protein (Ref. 37). Although these studies measured protein levels, protein expression is known to correlate with gene amplification (detected by either molecular methods or FISH), and the results illustrate the important principle that molecular markers can predict response to treatment.

The prognostic significance of *P53* has been investigated in many studies worldwide, involving nearly 3000 breast cancer patients in total. These studies have shown that somatic mutations in *P53* represent independent markers of early relapse and death (summarised in Table 3). The relative risk (hazard) ratio of death in cases of breast cancer that are associated with somatic mutations in *P53* varied in the range of 1.5–23.6, whereas recurrence varied in the range of 1.9–4.7. Although these findings suggest that patients with mutations in *P53* might benefit from more intensive (or different) treatment, reports of the influence of mutations in *P53* on the response to treatment have been conflicting. In one study, adjuvant chemotherapy (especially with tamoxifen) in combination with radiotherapy was found to be of less value in tumours with mutations in *P53* (Ref. 38); whereas another study reported that local or regional radiotherapy for tumours that did not involve lymph nodes improved the survival only in patients with tumours that were positive for *P53* mutations (Ref. 39). Mutations in *P53* have also been linked to primary resistance to doxorubicin

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Table 3. Prognostic significance of *P53* mutations in primary breast cancers (tab003ccc)

Study	Mutation detection method[a] (and exons analysed, where appropriate)	Number of cases studied (median follow-up, in months)	Relative risk (hazard) ratio (95% CI)	
			Relapse	Death
Andersen et al, 1993 (Ref. 102)	CDGE Exons 5–8	163 (48)	2.2 (1.2–4.0)	2.9 (1.2–7.1)
Bergh et al, 1995 (Ref. 38)	Sequencing cDNA	316 (57)	NA	2.0 (1.0–3.90)
Berns et al, 1998 (Ref. 103)	SSCP Exons 5–8	222 (115)	1.6 (1.1–2.4)	1.5 (0.97–2.2)
Caleffi et al, 1994 (Ref. 104)	CDGE Exons 5–9	192 (48)	NA	Not significant
Elledge et al, 1993 (Ref. 105)	SSCP Exons 5–9	190 (71)	1.9 (1.0–3.5)	NA
Falette et al, 1998 (Ref. 106)	Sequencing Exons 2–11	113 (105)	NA	1.81 (0.99–3.30)
Iacopetta et al, 1998 (Ref. 107)	SSCP Exons 4–8	422 (74)	1.6 (1.1–2.5)	2.1 (1.3–3.5)
Kovach et al, 1996 (Ref. 108)	ddF Exons 2–11	90 (24)	4.7 (1.4–16)	23.4 (2.4–228)
Riou et al, 1993 (Ref. 109)	Sequencing cDNA	24 (54)	NA	8.6 (1.4–52.5)
Saitoh et al, 1994 (Ref. 110)	ddF Exons 2–11	52 (19)	Significant (p=0.05)	Significant (p=0.002)
Seshadri et al, 1996 (Ref. 111)	SSCP Exons 5–6	727 (66)	NA	2.4 (1.5–3.8)
Shiao et al, 1995 (Ref. 112)	SSCP Exons 5–8	92 (NA)	NA	5.6 (1.4–23.0)
Soong et al, 1997 (Ref. 113)	SSCP Exons 4–10	375 (57)	NA	2.5 (1.2–5.2)
Thorlacius et al, 1995 (Ref. 114)	CDGE Exons 5, 7, 8	109 (32)	NA	3.3 (1.6–6.7)

[a] CDGE = constant denaturing gel electrophoresis; cDNA = coding DNA; ddF = dideoxy fingerprinting; SSCP = single strand conformation polymorphism.
Abbreviations used: CI = confidence interval; NA = not available; p = p value.

therapy and early relapse in patients with breast cancers (Ref. 40).

The tumour suppressor gene *BRCA1* might also be of prognostic significance in human breast cancer. Breast cancers that occur in women with germline mutations in *BRCA1* have been shown to have the histopathological features that are typically associated with a poor prognosis (Refs 41, 42, 43, 44). However, the results of studies of clinical outcome have been conflicting: one recent study suggested a better prognosis for breast cancer associated

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with *BRCA1* mutations compared with sporadic breast cancers (Ref. 45). Another study showed that disease-free survival and overall survival were similar in sporadic and *BRCA1*-associated breast cancer (Ref. 46); whereas a third, small study found that overall survival was poorer in breast cancers that were associated with a mutation in *BRCA1* compared with cancers that had no mutations in *BRCA1* (Ref. 47).

Colorectal cancer

The importance of genetic alterations in predicting clinical outcome has also been extensively studied for human colorectal cancer, although the results of these studies have sometimes been conflicting. For example, allelic deletions (loss of heterozygosity) on chromosome 17p (that is the p arm of chromosome 17) are associated with metastasis to lymph nodes, microvascular invasion (Ref. 48) and poorer survival of the patient (Ref. 49). Similarly, allelic loss of the *DCC* locus (on chromosome 18q) is associated with liver metastasis (Ref. 50). However, the reduced survival that is associated with the allelic loss of chromosome 17p is not independent of *P53* mutations (Ref. 51). Although one study found allelic deletions at chromosome 18q to have no prognostic significance (Ref. 52), most studies have found that loss of heterozygosity on chromosome 18q predicts a reduced five-year survival in Stage II (Refs 53, 54, 55) and Stage III cancers (Ref. 53). Other chromosomal abnormalities that correlate with poor survival include allelic deletions (loss of heterozygosity) on chromosome 1p (Ref. 56).

Other molecular genetic markers that can predict the prognosis of colorectal cancer include mutations in the *P53*, *K-ras* and *L-myc* genes. Three studies have shown that mutations in *P53* are predictive of poor survival (Refs 51, 57, 58), and mutations in *K-ras* have been shown either to be more frequent in patients with recurrent disease (Ref. 59) or to be an independent marker for poor survival (Ref. 60), despite the fact that mutations in *K-ras* are not correlated with other characteristics that are known to be predictive of prognosis (Ref. 61). In addition, mutations in *K-ras* have been found to be a poor prognostic marker in Duke's Stage A and Stage B colorectal cancer (Ref. 62), and in Stage II but not Stage III tumours (Ref. 63). Rather unexpectedly, mutations in *K-ras* in both codon 12 and 13 of this gene have been reported in one study to be markers of long-term survival (Ref. 64).

Other cancers

The evidence for the importance of molecular markers in other cancers is also beginning to accumulate; mutations in the *P53* gene are associated with shorter survival in pancreatic adenocarcinoma (Ref. 65) and ovarian cancer (Ref. 66). In addition, the allelic loss of chromosome 18q is associated with poorer survival in squamous cell carcinomas of the head and neck (Ref. 67). In contrast, mutations in *P53* have been of uncertain prognostic significance in adult cerebral glioblastomas (Refs 68, 69). One recent study has shown that the presence of *P53* mutation(s) is a marker for better radiation response in glioblastomas, which results in significantly longer survival (Ref. 70). The relevance of mutations in *BRCA1* in ovarian cancer is also controversial: one study found that patients with ovarian cancer that were associated with mutations in *BRCA1* had a better prognosis than those patients with sporadic ovarian cancers not associated with *BRCA1* mutations (Ref. 71); however, a subsequent study found the reverse to be true (Ref. 72). Further prospective studies in carefully controlled case series are needed to provide definitive results.

Clinical implications/applications

Many researchers and oncologists believe that a greater understanding of the genetic bases for the pathogenesis of cancer is likely to bring clinical benefits. Genetic markers have the potential to provide both sensitive and specific tests, which can be used in the screening, early diagnosis, staging and surveillance of cancer. Although no one genetic marker is likely to be useful by itself, it is hoped that a more thorough and accurate definition of the genetic alterations that characterise common cancers, combined with technological advances that facilitate testing for multiple alterations, will provide a minimal set of molecular markers for effective screening of asymptomatic individuals. In addition, molecular fingerprinting will enable improved prediction of the likely behaviour of tumours, and a combination of molecular staging with molecular fingerprinting will provide benefits such as greater prognostic accuracy and improved decision making about treatment. It is likely that more general markers of genetic changes such as loss of heterozygosity, microsatellite instability

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and DNA methylation will help in developing diagnostic studies. Mutations in oncogenes, which require prior knowledge of the specific mutation, should prove to be extremely helpful in the staging and prognosis of cancer.

Research in progress and outstanding questions

Considerable research effort is continuing to elucidate the molecular genetic changes that are characteristic of cancer. Technical developments should improve the reliability of detecting specific markers in clinical specimens. Although there is enormous potential for the use of molecular genetics in the diagnosis and management of cancer, large-scale clinical trials are urgently needed to assess such techniques in a clinical setting.

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Features associated with this article

Tables

Table 1. Prevalence (%) of some common somatic genetic alterations in common sporadic cancers (tab001ccc).
Table 2. Studies that have used molecular genetic alterations to identify tumour cells in clinical samples (tab002ccc).
Table 3. Prognostic significance of *P53* mutations in primary breast cancers (tab003ccc).

Schematic figures

Figure 1. Sequential genetic changes that are associated with dysplasia and malignant neoplasia of the colon (fig001ccc).
Figure 2. The effects of microsatellite instability (MIN) and loss of heterozygosity (LOH) on $(CA)_n$ dinucleotide tandem repeats (fig002ccc).
Figure 3. Detection of genetic changes in neoplastic tissue by the analysis of $(CA)_n$ dinucleotide tandem repeats using the polymerase chain reaction (PCR) (fig003ccc).

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Further reading, resources and contacts

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The Cancer Genome Anatomy Project (CGAP) is an interdisciplinary programme to establish the information and technological tools needed to decipher the molecular anatomy of a cancer cell.
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Cancer Rates and Risks is an on-line publication that provides international cancer incidence and mortality rates in a series of charts and graphs. It also includes information on cancer risk factors (e.g. alcohol, pesticides) and risks for major cancers. It is produced by the National Cancer Institute of the US National Institutes of Health.
http://rex.nci.nih.gov/NCI_Pub_Interface/raterisk/index.html

The journal Seminars in Cancer Biology covers a different topic of cancer biology in each issue. Previous topics include: tumour suppressor genes, oncogenes and differentiation.
http://www.idealibrary.com/cgi-bin/links/toc/se
UK mirror site: http://www.europe.idealibrary.com/cgi-bin/links/toc/se/

OncoWeb is a free, on-line educational resource and information service for professionals working in the oncology field. This resource is provided by the European School of Oncology, and Greenwich Medical Online. The site includes the home page of the European Association for Cancer Research, and also the searchable and browsable START database of oncology treatment. Other information provided includes conference details, case studies and links to related cancer sites. The content of the pages is reviewed by oncology specialists on the OncoWeb Editorial Board.
http://www.oncoweb.com

The CANCERLIT site provides a full searching facility for the CANCERLIT bibliographic database, from 1993 to present. The National Cancer Institute's International Cancer Information Center (ICIC) produces the CANCERLIT database by extracting the majority of relevant citations from MEDLINE. This core information is supplemented with additional citations of books, meeting abstracts, theses and other publications. In addition, the ICIC creates abstracts in CANCERLIT for selected citations that do not have author abstracts. The database is updated monthly. CANCERLIT citations and abstracts are also searchable from the CancerWEB site, but only those added during the previous 12 months.
http://www.graylab.ac.uk/cancernet/cancerlit/index.html
UK mirror site: http://cnetdb.nci.nih.gov/cancerlit.shtml

Human Genome Project: from Maps to Medicine is a short, illustrated guide to the Human Genome Project and its applications to medicine, published by the National Center for Human Genome Research (part of the US National Institutes of Health). This document contains a basic introduction to genetics and the Human Genome Project, as well as a case study on hereditary colon cancers.
http://www.nhgri.nih.gov/Policy_and_public_affairs/Communications/Publications/Maps_to_medicine/index.html

Breast Cancer Information Core is an open-access on-line database of breast cancer mutations. It also provides links to other breast-cancer-related sites.
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