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Cancer genetics and their application to individualised medicine

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Abstract

One of the great challenges of basic research is to translate scientific discoveries into the improved treatment of patients. For colorectal cancer, our increased understanding of the molecular aetiology of the disease has not yet been paralleled by an improvement in patient care. However, several new approaches are on the verge of clinical implementation. Technical advances such as real-time polymerase chain reaction (PCR) and microarray techniques coupled to insight in the molecular pathways in colorectal cancer makes it possible to develop new clinical tools for the diagnosis, classification and treatment of patients. The ultimate goal of the incorporation of cancer genetics into the clinical treatment of patients is individualised medicine; therapeutic strategies based on the molecular taxonomy of tumours and individually constructed for each patient. © 2002 Published by Elsevier Science Ltd.

1. Introduction

Probably all disease states except trauma are influenced by genetic factors. In the last decades of the 20th century, all avenues of biomedical research have led to the gene. The human genome contains all of the information necessary from conception until death. The Human Genome Project (HGP) has resulted in the sequencing of the entire human genome [1]. This achievement will dramatically accelerate the development of new strategies for the diagnosis, prevention and treatment of disease. Especially for common complex diseases such as cancer, genetic differences may contribute to the risk of contracting the disease, the clinical course of the disease and responsiveness to different treatments [2]. Endeavours such as the HGP and technical advances in molecular biology are expected to have a revolutionary effect on the treatment of cancer. The increasing understanding of complex molecular pathways involved in cancer will shift clinical practice from empirical treatment to treatment based on a molecular taxonomy of disease [3].

The need for new diagnostic and therapeutic strategies is particularly evident in oncology. Classification of tumours into pathogenetical subtypes with distinct clinical courses enables clinicians to target therapy. For colorectal cancer, the Dukes' classification and TNM staging system remain the golden standards and rely entirely on the morphological appearance of the tumour. Tumours with similar histopathological characteristics, however, may have distinct clinical outcomes and responsiveness to therapy. Different individual genetic make-up for the metastasising potential of each tumour will determine the need for specific adjuvant treatment protocols. Within the current classification systems, important subclasses are likely to exist, but have yet to be defined using systematic and unbiased approaches.

The mainstay of colorectal cancer therapy remains wide *en-bloc* resection. Although curative in early stage cancer, many patients present themselves with advanced stage disease where (micro) metastatic disease is already present. While the clinical and pathological heterogeneity of colorectal cancer has long been recognised, standard therapy, however, remains non-specific; cytotoxic drugs that are effective only in some patients, yet cause side-effects in most.

In this paper, we describe the cancer genetics of colorectal tumours and how this knowledge can be translated into more precise and patient-tailored clinical applications. The recently developed techniques for genomewide gene expression analysis and the anticipated role in clinical oncology are discussed in the second part of this paper.

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2. Colorectal cancer genetics

Colorectal carcinoma develops along a 'classical' pathway outlined by Fearon and Vogelstein [4]. The genes responsible for familial adenomatous polyposis (FAP) and hereditary non-polyposis colorectal cancer (HNPCC) have been identified. Inherited mutations in the *APC* gene (FAP) and DNA mismatch repair (MMR) genes, e.g. *hMSH2* and *hMLH1* (HNPCC) are responsible for these cancer syndromes [5]. Both pathways can also be discerned in sporadic cases, where mutations in the *APC* tumour suppressor gene initiates the majority of colorectal cancers. The selective force behind the 'gatekeeper' role of APC is the abrogation of the normal Wnt-signalling pathway [6]. Wild-type APC acts in a multiprotein complex that degrades the oncogene β-catenin. Loss of APC function leads to the stabilisation of β-catenin and formation of constitutive complexes with T cell factor (Tcf)-4 [7]. These complexes are transported to the nucleus. Here, oncogenic Wnt target genes, such as *c-myc*, *c-jun* and *cyclin-D1*, are activated [8]. Interestingly, in tumours that lack *APC* mutations, activating mutations of β-catenin have been found, illustrating the importance of the Wnt signalling pathway in colorectal cancer initiation [9].

Further development from adenoma to carcinoma involves several distinct genetic steps. Somatic mutations at codons 12 and 13 of the K-*ras* oncogene are frequently present in adenomas and carcinomas [10]. K-*ras* is involved in signal transduction. Somatic mutations in the tumour suppressor gene *TP53* occur in approximately 60% of colorectal cancers [11]. Wild-type p53 function is associated with cell cycle progression, apoptosis and protection against DNA damage. Chromosome 18q is frequently lost in colorectal tumorigenesis and usually involves the *DCC* gene (deleted in colorectal cancer), but several other genes, including *Smad4*, in the same region are also implicated [12]. The biological consequence of loss of the *DCC* product, the Netrin-1 receptor, is not known. Recent studies suggest that inability of the DCC product to bind to Netrin-1 impairs apoptosis, which could result in a resistance to chemotherapy [13].

3. Diagnosis

Many molecular alterations involved in the initiation and progression of colorectal cancer have been characterised. It is now opportune to use this knowledge for the clinical benefit of cancer patients. A clear example of the rapid pace with which molecular biology enters clinical practice is the way in which patients with hereditary colorectal cancer (FAP and HNPCC) are diagnosed. Before the identification of the many genes that underlie these hereditary cancer syndromes, family history was the only way to identify presymptomatic individuals at high risk. Genetic testing for germline *APC* mutations is now widely used to identify within the FAP families, the individuals who are at high risk [14]. Immunohistochemical tests for *h*MSH2 and *h*MLH1, microsatellite instability (MSI) testing and screening for germline mutations in the mismatch repair genes, are options available to test possible HNPCC patients. The combination of clinical findings and genetic testing in families with a clustering of colorectal cancer has been reported as a powerful strategy in families suspected to have HNPCC [15].

In sporadic tumours, specific genetic alterations are used for the diagnosis of micrometastatic disease. Detection of somatic mutations in the *TP53* tumour suppressor gene and K-*ras* oncogene from the primary tumour in lymph node DNA is one method to detect minimal residual disease. Another method is reverse transcriptase-polymerase chain reaction (RT-PCR) of carcino-embryonic antigen (CEA) from RNA extracted from lymph nodes. Using that method, we have reported that micrometastases present in lymph nodes from stage II patients may identify a subgroup with poor prognosis. Micrometastases significantly reduced 5-year survival rates from 91% in patients without micrometastases to 50% in patients with micrometastatic disease in one or more lymph nodes [16]. Early diagnosis of disseminated disease in stage II colorectal cancer patients is a strong argument for selective use of adjuvant therapy.

4. Stratification

The need for new prognostic markers that will facilitate the identification of patients best treated with a more tailored therapy is clear. Currently, stage III colorectal cancer patients are all treated with adjuvant chemotherapy. However, the study of Moertel indicates that after surgery alone 45% of patients are still alive after 5 years compared with 65% in the chemotherapy group [17]. These numbers indicate that nearly half of all patients who will receive chemotherapy would have been cured without it and 35% will die regardless of therapy. The genetic changes commonly observed in sporadic colorectal cancer are frequently studied for their use as predictive markers in an attempt to provide the clinician with a rationale for therapeutic intervention. Many molecular alterations have the potential to predict survival after chemotherapy [18–20].

4.1. TP53

The prognostic value of *TP53* has been analysed extensively in many studies (reviewed in Ref. [21]). The results from those studies are conflicting. p53 Protein accumulation has been reported to correlate with

patient survival in some studies, while no correlation could be found in other studies.

In 1995, Goh and colleagues described that patients with *TP53*-mutated tumours had a significantly poorer prognosis that patients whose tumour were *TP53* wild-type [22]. Interestingly, they also found a correlation of the mutational status of *TP53* and response to post-operative chemotherapy. Not all point mutations being equally clinically significant, point mutations within the four conserved regions of the gene tended to be more aggressive. Essentially the same findings were reported by a group from Oslo [23]. In a series of 220 patients, they confirmed the poor prognosis of patients with *TP53* mutated tumours and mutations within specific domains having a more profound effect. Furthermore, Tortola and colleagues found that *TP53* mutations alone or in combination with K-*ras* mutations are correlated with a worse outcome [24]. In a subanalysis of patients undergoing a radical resection, this prognostic significance was lost. They concluded therefore that mutation analysis of *TP53* and K-*ras*, although helpful in the assessment of prognosis, is of no benefit as regards routine use in a clinical setting.

Many studies have attempted to find a correlation between the presence of *TP53* mutations and the response to chemotherapy. Ahnen and colleagues reported a better 7-year survival rate after 5-fluorouracil (5-FU) treatment in patients who had tumours without increased p53 protein levels than in those who had tumours with increased p53 [25]. However, a recent study that analysed patients enrolled in the same clinical trial could not confirm these findings. Immunohistochemical staining of p53 showed no correlation with prognosis or therapeutic response [18]. These discrepant results in patients from the same clinical trial emphasise the need for a standardisation and validation of the methodology, patient selection and interpretation of clinical data before any prognostic marker can be routinely used.

4.2. LOH18q

In 1994, Jen and colleagues showed that loss of heterozygosity (LOH) of chromosome 18q was associated with an adverse prognosis [26]. Five-year survival rates in stage II and III patients were significantly decreased in patients with LOH. Subsequent studies confirmed the association of 18q LOH with survival [27–29]. A recent paper by Watanabe and colleagues evaluated the prognostic significance of 18q LOH in 460 stage III patients from two randomised trials of adjuvant chemotherapy for colon cancer [18]. Five-year survival after fluorouracil-based chemotherapy was 74% in patients whose tumour had retained chromosome 18q versus 50% in patients with a loss of 18q. However, since all patients had received some kind of 5-FU-based chemotherapy, it is not known whether this marker reflects resistance or sensitivity to chemotherapy. Considering the wealth of data confirming the prognostic significance of the loss of 18q, it would be worthwhile to test this marker in a prospective randomised trial.

4.3. Microsatellite instability

Tumours with high levels of microsatellite instability (MSI) are characteristic for patients with HNPCC. In sporadic tumours, MSI occurs in 10–15% of cases and is frequently caused by promoter methylation of the mismatch repair gene, *hMLH1* [30]. As a consequence, genes containing simple repeat sequences such as *TGF-betaRII* or *BAX* are often mutated in these tumours. Several studies indicate that MSI is an independent prognostic factor. Halling and colleagues report in a retrospective study of over 500 patients, a decrease in survival from 74% in stage III patients with MSI to 55% in patients without MSI [31]. The above-mentioned paper from Watanabe did not find a statistical significant correlation of MSI and survival in stage III patients (68% versus 56%; $P=0.20$). However, when the analysed tumours with MSI were also examined for mutations in the *TGF-betaRII* gene, survival dropped significantly from 74% in patients with a mutation to 46% in patients with wild-type copies of the gene.

Despite a 2–3-fold higher mutation rate in tumours with a mismatch repair deficiency, patients with these tumours tend to have an increased relative survival. The mechanisms underlying this phenomenon are not known. It is speculated that the mutator phenotype targets simple repeat sequences in genes that are essential for cell survival. The high mutation rate in these tumours thus acts as a barrier for tumour progression.

Again, this kind of prognostic information may allow the stratification of patients within one tumour stage to target therapy and to design future adjuvant trials.

5. Therapy

All factors of the genetic pathway in colorectal cancer are potentially useful as targets for therapy. Considering the pivotal role of the Wnt pathway, components could be used to design new drugs. Currently, researchers are working on low-molecular-weight substances that specifically block the Tcf/beta-catenin interaction. This would block transcription of downstream targets and consequently prevent tumour initiation or progression. Recent progress in breast cancer research revealed a molecular explanation for the pattern of site-specific metastases. Müller and colleagues found that a leucocyte chemoattractant receptor (CXCR4) is expressed in breast cancer cells and its ligand (CXCL12) is expressed in lymph nodes, bone marrow and lung, to which breast cancer often spreads [32]. In an immunodeficient mouse

model, they showed that blockade of CXCR4 reduced the number of metastases. Development of small-molecule antagonists of CRCX4 (and perhaps other chemokines in other cancers) could be used to prevent secondary metastases [33].

The tremendous progress in our knowledge of pivotal pathways in colorectal oncogenesis may be used to design different therapies for tumours with different genetic make-up. The large number of genes that are involved in colorectal cancer can now be studied for their role in drug activity, toxicity and metabolism.

6. Microarray technique

Traditionally, the elucidation of genes involved in carcinogenesis was accomplished one gene at a time. The recent development of cDNA microarrays enables the parallel monitoring of expression levels of thousands of genes. Furthermore, construction of genomewide expression patterns of all tissues and disease states will be possible in the near future. This will undoubtedly have an enormous impact on our understanding of cell biology, but may also prove an important link between molecular genetics and clinical medicine.

Current cDNA microarray protocols are based on the Southern blot technique in which labelled nucleic acid molecules are hybridised to complementary nuclear acid molecules attached to a solid surface such as glass (Fig. 1). Technical innovations such as miniaturisation and fluorescence-based detection, greatly enhances the throughput.

A microarray consists of thousands of small spots of multiple copies of amplified cDNA spotted on a glass microscopic slide. Each spot represents a unique sequence from a named gene or expressed sequence tag (EST). An EST is a sequence from a cDNA clone that corresponds to a messenger RNA molecule (mRNA). They allow the identification of coding regions in genomewide sequences and are used to identify new genes [34]. Currently, over 1 million ESTs are deposited in large data banks such as dbEST (www.ncbi.nlm.nih.gov/dbEST/index.html). Considering estimates of the total number of human genes, approximately 40 000, there must be a considerable overlap. At the National Center for Biotechnology Information of the National Institute of Health in the US, an automated process called UniGene compares ESTs and assembles overlapping sequences. From this non-redundant set, many laboratories have selected clones for the production of microarrays. Once the selected cDNAs are amplified, these products are spotted onto microscope slides using commercially available robots. Ultraviolet light then cross-links these probes to the glass slides. One slide can hold up to 10 000 probes.

As a target for analysis, total RNA or mRNA from two cell populations is used (e.g. cell lines, clinical samples and

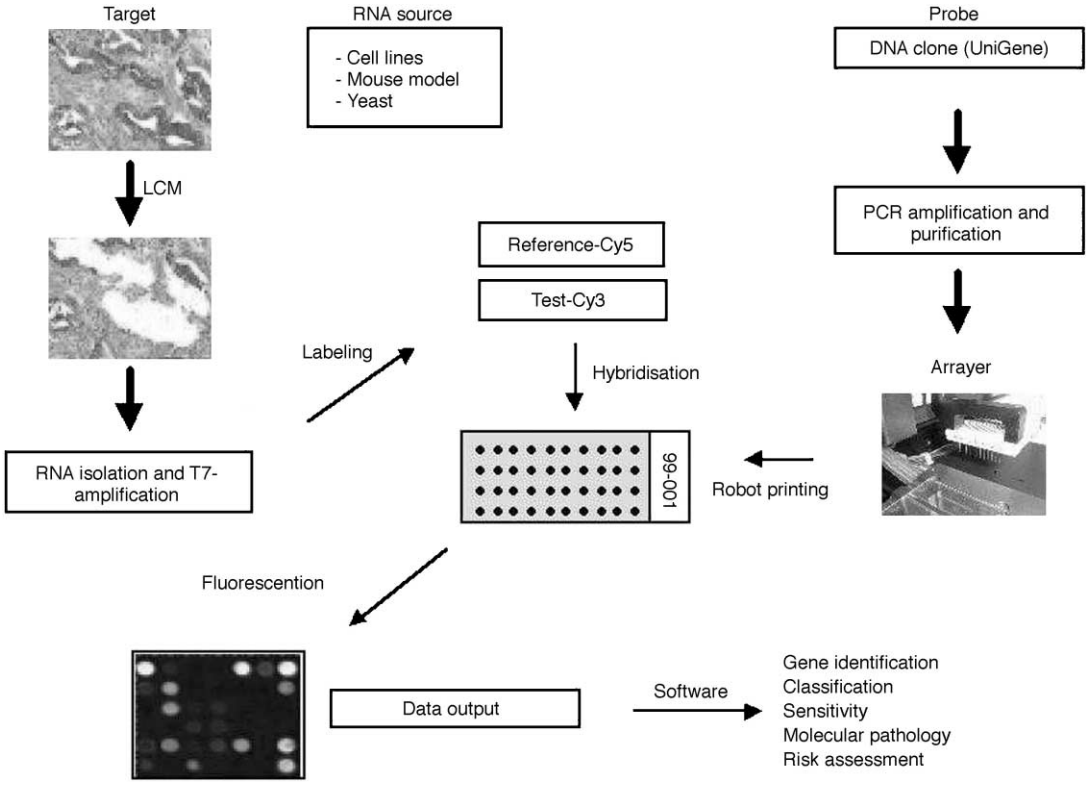

Fig. 1. Flow chart of the microarray technique. Hybridisation of labelled RNA from any given source to probes on a array yields information of the relative abundance of transcripts between samples. These large-scale gene expression data can be organised using specific software tools.

animal models). Current protocols cannot measure absolute amounts of mRNA copy numbers present in one cell. This technique measures the relative abundance of transcripts between samples. Unfortunately, these protocols require large amounts of RNA. Typically, 50–100 μg of total RNA (or 2–5 μg when mRNA is used) is needed. Especially in clinical samples, where material is often limited or cells of interest are few, this is a serious drawback. Enzymatic amplification protocols have been developed to bypass this problem and RNA from a few hundreds of cells is sufficient for gene expression analysis [35]. Obviously, amplification may introduce an unknown bias. Preferential amplification of specific sequences or enzymatic mismatches may occur and these data should be interpreted with care.

Fluorescent marker dyes such as Cy3 and Cy5 are incorporated into target cDNA. The labelled cDNA from the two cell populations of interest are mixed with a labelled control sample and hybridised to the probes on the glass slides. The array is scanned using confocal laser microscopy. After excitation and emission of fluorescence, signals can be measured and displayed. This results in a matrix of thousands of green, red and yellow spots. When, for example, a gene is equally expressed in test and control samples, both the red and green fluorescent signals will be equally strong and will be visualised as a yellow dot (Fig. 2). Consequently, in the case of differential expression, the red to green ratio will shift. Following hybridisation and scanning, large amounts of data are available for processing. A variety of software tools are available which can help to measure fluorescent signal ratios, exclude artifacts and normalise data.

The amount of applied target, extent of target labelling, hybridization efficiency and other variables may vary significantly between single experiments. To accurately compare measurements from different experiments, data need to be 'normalised' by the inclusion of a distinct set of genes in the array of which ubiquitous expression is expected in all cells such as the selection of the so-called 'house-keeping' genes. For closely related samples, the expression of many genes will not vary significantly, and in this case, global normalisation is possible. Explicit methods have been developed which make use of subsets of genes for normalisation, and extract from the variance of this subset statistics for evaluating the significance of the observed changes in the complete data-set [36].

7. Gene expression patterns in human tumours

Microarray analysis is expected to play a major role in the implementation of genome-based, tailor-made medicine. Through the evaluation of large-scale gene expression profiles, tumours can be classified and important molecular pathways can be discerned. One of the first studies that used gene expression data to supplement standard cancer histopathology studied the clinical heterogeneity of diffuse large B-cell lymphoma (DLBCL), the most common subtype of non-Hodgkin's

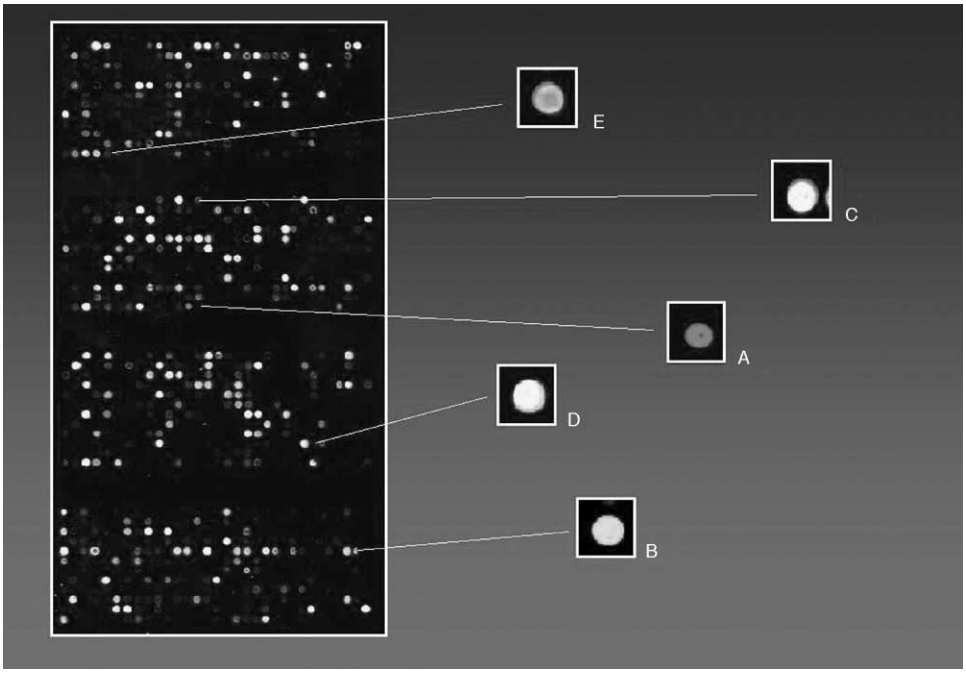

Fig. 2. Detail of a microarray. In this experiment, RNA from a rectum tumour (labelled red) is compared with RNA from a pool of normal mucosa (labelled green). Gene A shows a bright red spot indicating that this gene is preferentially expressed in carcinoma. In contrast, gene B is only expressed in normal mucosa and its expression is consequently suppressed during tumorigenesis. In the case of equal expression, a yellow spot will show (gene C). Genes D and E are intermediates.

lymphoma. Alizadeh and colleagues could differentiate between DLBCLs with a good and bad prognosis on the basis of cDNA microarray analysis [37]. This paper is a good example of how new technology may improve the diagnosis of cancer. When more is learned about the molecular pathways in the different cancer subtypes, more molecularly targeted therapies can be developed based on the biology of the disease.

Recently, two papers reported expression profiles of colorectal normal tissue, adenomas and adenocarcinoma. Notterman and colleagues used a commercially available oligonucleotide array (GeneChip, Affymetrix) for the molecular classification of the colorectal tumours [38]. In this study, only 1.8% of 4000 transcripts were differentially expressed in the tumours and normal tissue. This low number is likely to reflect the close relationship of the samples studied. A two-way hierarchical clustering algorithm successfully distinguished adenoma from adenocarcinoma and normal tissue. Their study showed that molecular classification of solid tumours can be performed on the basis of genomewide expression profiling. Another study from Kitahara and colleagues used a microarray consisting of 9.216 cDNAs to examine changes in expression patterns related to colorectal carcinogenesis [39]. They could discern different patterns of expression in normal and neoplastic colon. Both studies are very similar, i.e. they want to elucidate genes that are involved in the transition from normal colon epithelium to adenocarcinoma. Using large-scale gene expression analysis, they both find one to two hundred genes that are implicated in tumour progression. However, only three genes (*pyrroline 5-carboxylate reductase* mRNA, *BENE* and *carbonic anhydrase II*) were found to be differentially expressed in adenocarcinomas and normal colon in both studies. This remarkable lack of overlap probably reflects differences in the materials and methodology, but is a major point of concern. Both studies differ in the construction of the cDNA array, the number of patients, the software used for analysis, etc. Important clinically relevant answers, however, will come from the comparison of different experiments and the analysis of large numbers of patients. Meta-analysis of gene expression levels requires a common standard for comparing measurements and the general availability of raw data-sets [40]. In addition, a uniform format for display and storage of data is needed. Such a database should contain a definition of the sequences used in the array, a thorough description of the source and preparation of targets, details of methodology and a detailed description of patient-related variables and follow-up [41].

Another clinically interesting study reported gene expression in highly metastatic melanoma cells [42]. An *in vivo* selection scheme to isolate melanoma cells with high metastatic potential from their non-metastatic counterparts was used. Next, microarray analysis revealed genetic profiles that correlated with the progression to a metastatic phenotype. Among the genes that showed an enhanced expression in the metastatic cells were RhoC, fibronectin and thymosin β. This kind of data can be used to predict the metastatic potential of tumours and may yield new therapeutic targets for adjuvant therapy.

Our group studied the role of genomewide gene expression analysis in the selection of high-risk rectal cancer patients (data not shown). In a case-control study, we extracted tumour RNA from 23 patients with or without liver metastasis. All patients were included in a randomised clinical trial in which surgery and pathological examination were uniform and quality controlled. Our data indicate that patients with stage II/III rectal cancer who develop metastasis after a curative (R0) resection can be discriminated from patients without recurrent disease on the basis of differentially expressed genes. Our analysis included 9216 distinct genes and ESTs. Only 35 of the genes studied showed differential expression in the metastatic rectal tumours. This relatively small number indicates the close relationship of the samples. It is possible that they are important in the actual process of metastasis. Others, using various types of cDNA microarray also reported small percentages (0.2–10%) of differentially expressed genes in closely related samples [38].

8. Pharmacogenomics

Another field of research that uses large-scale gene expression analysis focuses on variations in the drug response. In colorectal cancer, particularly in metastatic disease, 5-FU-based regimens are only marginally effective. A predictive test for drug response would allow the clinician to offer 'tailor-made' therapy to an individual patient. Two publications in *Nature Genetics* report the exploration of differentially expressed genes among the 60 cell lines used in the National Cancer Institute's screen for anticancer drugs [43,44]. These studies show how variations in the transcript levels of particular genes relate to mechanisms of drug sensitivity and resistance. This kind of information may provide a means to monitor both the desired effects of drugs and to obtain warnings of unexpected adverse effects. It is anticipated that pharmacogenomics will eliminate many uncertainties of current therapy for cancer and provide clinicians with targets for innovative treatment protocols. For example, Esteller and co-workers found that the clinical response of gliomas to alkylating agents correlated well with inactivation of the DNA-repair gene *MGMT* through methylation [45]. Using this kind of data, genetic alterations can help to match the best therapy to the right patients.

Whether these data can be used to guide the clinician in diagnosing and treating colorectal cancer patients remains unclear. Naturally, there are some drawbacks

to large-scale gene expression analysis. Solid tumours are heterogeneous with regard to the metastatic potential of different cancer cells within one tumour. It is therefore possible that the expression of genes responsible for metastasis will remain undetected. It would require a three-dimensional approach to characterise an entire tumour. Secondly, a causative role of genes that are identified as discriminative for metastasis has to be determined. It is entirely possible that many differentially expressed genes are an indirect consequence of the altered phenotype of cancer cells. The elevation of many genes in cancer cells may be a secondary effect of the enhanced proliferative state of a tumour which requires a higher expression of genes related to cell growth, nucleic acid synthesis and cellular metabolism. Next, newly identified markers need to be validated in large groups of patients before they can be of relevance to clinical oncology. Microarray data are therefore by no means endpoints. Rather, they are hypothesis-driven starting-points for the development of new therapeutic strategies.

9. Statistical considerations

Considering the wealth of data that has appeared in the literature, the use of molecular markers and gene expression profiles in routine clinical practice seems on the verge of implementation. Indeed, impeding issues such as high costs and the complexity of the techniques are easily surmountable. However, data from molecular studies need to be confirmed in large clinical trials. A shift towards individualised, genome-based diagnosis, classification and treatment of cancer will exponentially enlarge the amount of subgroups of patients in which new therapeutic strategies need to be tested. For example, if for TNM stage II colorectal cancer patients a genetic profile can be discerned in 25% of patients that accurately predicts the likelihood of liver metastases. Moreover, further genetic association studies indicate a polymorphic marker in a drug-metabolising enzyme that correlates with drug response, and the proportion of patients having that allele is 20%. To test whether chemotherapy can improve prognosis in stage II patients with a high chance of liver metastases and the right polymorphic marker these patients should be enrolled in a two-arm randomised trial. In The Netherlands, 3000 stage II patients are diagnosed each year. In our example, it would mean that only 150 patients are eligible for randomisation. To conduct a randomised trial with sufficient statistical power for unequivocal conclusions one to two thousand patients need to be included. Even in a nationwide endeavour, this would seem impossible. This example argues strongly for the need to construct tissue banks. Fresh frozen and paraffin-embedded tissue should be available for molecular studies from all patients participating in large multicentre trials. Data derived from studies with uniform treatment groups will allow physicians to evaluate genetic prognostic markers for patients with colorectal cancer [46].

10. Future oncology

In his 1999 Shattuck Lecture, Francis S. Collins, from the National Human Genome Research Institute, NIH, envisioned a hypothetical patient in 2010 [2]. Analogous to that lecture, we would also like to present a hypothetical future patient:

A 65-year-old male patient is discussed in the weekly oncology meeting. This patient was diagnosed with left-sided colon carcinoma and had undergone a left-sided hemicolectomy with primary anastomosis. The results of the microarray analysis of his tumour were returned from the regional test centre within 1 week with the following information. The genetic profile of all the pathways involved in metastases revealed an 80% chance of contracting liver metastases within 5 years. The predicted response to 5-FU-based adjuvant therapy is poor, as indicated by a decreased expression of genes involved in fluoropyrimidene metabolism such as thymidylate synthase and dihydropyrimidine dehydrogenase. Furthermore, genetic variants of drug metabolising enzymes present in the patients genome make him susceptible to adverse side-effects. Therefore, after extensive counselling, the patient decides against standard chemotherapy. Specific expression patterns of genes involved in angiogenesis offer the possibility for an alternative approach. The field of pharmacogenomics has blossomed and a wide range of anti-angiogenic drugs are available. Further analysis shows high expression of some downstream targets of beta-catenin and a specific low-molecular weight blocker of the Tcf/beta-catenin is offered to the patient as well. The patient is willing to accept the side-effects of the offered regimen since he has learned that his genetic profile shows a reduced risk of coronary artery disease and Alzheimer's disease. Therefore, he has many high quality years to potentially live for.

Although at the present moment purely theoretical, this would be an exciting situation: therapeutic strategies based on the molecular taxonomy of tumours and individually constructed for each patient. Considering the pace of development, it even seems likely that it will affect the current generation of physicians treating patients with cancer in their busy everyday practices.

References

1. Lander ES, Linton LM, Birren B. Initial sequencing and analysis of the human genome. *Nature* 2001, **409**, 860–921.
2. Collins FS. Shattuck Lecture—Medical and societal consequences of Human Genome Project. *N Eng J Med* 1999, **341**, 28–37.

3. Golub TR. Genome wide views of cancer. *N Eng J Med* 2001, **344**, 601–602.
4. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell*, 759–767.
5. Kinzler WK, Vogelstein B. Lessons from hereditary colorectal cancer. *Cell* 1996, **87**, 159–170.
6. Polakis P. The adenomatous polyposis coli (APC) tumor suppressor. *Biochem Biophys Acta* 1997, **1332**, F127–F147.
7. Korinek V, Barker N, Morin PJ, *et al*. Constitutive transcriptional activation by a beta-catenin-Tcf complex in $APC^{-/-}$colon carcinoma. *Science* 1997, **275**, 1784–1787.
8. Behrens J. Control of beta-catenin signaling in tumor development. *Ann N-Y Acad Sci* 2000, **910**, 21–35.
9. Ilyas M, Tomlinson IPM, Rowan A, *et al*. Beta-catenin mutations in cell lines established from human colorectal cancer. *Proc Natl Acad Sci USA* 1997, **94**, 10330–10334.
10. Shibata D, Schaeffer J, Li ZH, *et al*. Genetic heterogeneity of the c-K-ras locus in colorectal adenomas but not in adenocarcinomas. *J Natl Cancer Inst* 1993, **85**, 1058–1063.
11. Nigro JM, Baker SJ, Preisinger AC, *et al*. Mutations in the p53 gene occur in diverse human tumour types. *Nature* 1989, **342**, 705–708.
12. Takaku K, Oshima M, Miyoshi H, *et al*. Intestinal tumorigenesis in compound mutant mice of both Dpc4 (Smad4) and Apc genes. *Cell* 1998, **92**, 645–656.
13. Mehlen P, Rabizadeh S, Snipas SJ, *et al*. The DCC gene product induces apoptosis by a mechanism requiring receptor proteolysis. *Nature* 1998, **395**, 801–804.
14. Powell SM, Petersen GM, Krush AJ, *et al*. Molecular diagnosis of familial adenomatous polyposis. *N Eng J Med* 1993, **329**, 1982–1987.
15. Wijnen JT, Vasen HFA, Meera Khan P, *et al*. Clinial findings with implications for genetic testing in families with clustering of colorectal cancer. *N Eng J Med* 1998, **339**, 511–518.
16. Liefers GJ, Cleton-Jansen AM, van de Velde CJH, *et al*. Micrometastases and survival in stage II colorectal cancer. *N Eng J Med* 1998, **339**, 223–228.
17. Moertel CG, Fleming TR, MacDonald JS, *et al*. Fluorouracil plus levamisol as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. *Ann Intern Med* 1995, **122**, 321–326.
18. Watanabe T, Tsung-Teh W, Catalano PJ, *et al*. Molecular predictors of survival after adjuvant chemotherapy for colon cancer. *N Eng J Med* 2001, **344**, 1196–1206.
19. Hemminki A, Mecklin JP, Järvinen H, *et al*. Microsatellite instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. *Gastroenterology* 2000, **119**, 921–928.
20. Elsaleh H, Joseph D, Grieu F, *et al*. Association of tumour site and sex with survival benefit from adjuvant chemotherapy in colorectal cancer. *Lancet* 2000, **355**, 1745–1750.
21. McLeod HL, Murray GI. Tumour markers of prognosis in colorectal cancer. *Br J Cancer* 1999, **79**, 191–203.
22. Goh HS, Yao J, Smith DR. p53 point mutation and survival in colorectal cancer patients. *Cancer Res* 1995, **55**, 5217–5221.
23. Børresen Dale AL, Lothe RA, Meling GI, *et al*. Tp53 and long-term prognosis in colorectal cancer—mutations in 13 zinc-binding domain predict poor survival. *Clin Cancer Res* 1998, **4**, 203–210.
24. Tortola S, Marcuello E, Gonzales I, *et al*. p53 and K-ras gene mutations correlate with tumor aggressiveness but are not routine prognostic value in colorectal cancer. *J Clin Oncol* 1999, **17**, 1375–1381.
25. Ahnen DJ, Feigl P, Quan G, *et al*. Ki-ras mutation and p53 overexpression predict the clinical behavior of colorectal cancer: a Southwest Oncology Group study. *Cancer Res* 1998, **58**, 1149–1158.
26. Jen J, Kim H, Piantadosi S, *et al*. Allelic loss of chromosome 18q and prognosis in colorectal cancer. *N Eng J Med* 1994, **331**, 213–221.
27. Ogunbiyi OA, Goodfellow PJ, Herfarth K, *et al*. Confirmation that chromosome 18q allelic loss in colon cancer is a prognostic indicator. *J Clin Oncol* 1998, **16**, 427–433.
28. Martinez-Lopez E, Abad A, Font A, *et al*. Allelic loss on chromosome 18q as prognostic marker in stage II colorectal cancer. *Gastroenterology* 1998, **114**, 1180–1187.
29. Shibata D, Reale MA, Lavin P, *et al*. The DCC protein and prognosis in colorectal cancer. *N Eng J Med* 1996, **335**, 1727–1732.
30. Kane MF, Loda M, Gaida GM, *et al*. Methylation of the hMLH1 promotor correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997, **57**, 808–811.
31. Halling KC, French AJ, McDonnel SK, *et al*. Microsatellite instability and 8p allelic imbalance in stage B2 and C colorectal cancers. *J Natl Cancer Inst* 1999, **91**, 1295–1303.
32. Müller A, Homey B, Soto H, *et al*. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001, **410**, 50–56.
33. Murphy PM. Chemokines and the molecular basis of cancer metastasis. *N Eng J Med* 2001, **345**, 833–835.
34. Adams MD, Kelley JM, Gocayne JD, *et al*. Complementary DNA sequencing: expressed sequence tags and Human Genome Project. *Science* 1991, **252**, 1651–1656.
35. Eberwine J. Amplification of mRNA populations using aRNA generated from immobilized oligo(dT)-T7 primed cDNA. *Biotechniques* 1996, **20**, 584–591.
36. Eisen MB, Spellman PT, Brown PO, *et al*. Cluster analysis and display of genome-wide expression patterns. *Proc Natl Acad Sci USA* 1998, **95**, 14863–14868.
37. Alizadeh AA, Eisen MB, Davis RE, *et al*. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000, **403**, 503–511.
38. Notterman DA, Alon U, Sierk AJ, *et al*. Transcriptional gene expression profiles of colorecal adenoma, adenocarcinoma, and normal tissue examined by oligonucleotide arrays. *Cancer Res* 2001, **61**, 3124–3130.
39. Kitahara O, Furukawa Y, Tanaka T, *et al*. Alterations of gene expression during colorectal carcinogenesis revealed by cDNA microarrays after laser-capture microdissection of tumor tissue and normal epithelia. *Cancer Res* 2001, **61**, 3544–3549.
40. Hollon T. Comparing microarray data: what technology is needed? *J Natl Cancer Inst* 2001, **93**, 1126–1127.
41. Liefers GJ, Tollenaar RAEM, Nakamura Y, *et al*. Genetic cancer syndromes and large scale gene expression analysis: applications in surgical oncology. *Eur J Surg Oncol* 2001, **27**, 343–348.
42. Clark EA, Golub TR, Lander ES, *et al*. Genomic analysis of metastases reveals an essential role for RhoC. *Nature* 2000, **406**, 532–535.
43. Ross DT, Scherf U, Eisen MB, *et al*. Systematic variation in gene expression patterns in human cancer cell lines. *Nature Genet* 2000, **24**, 227–235.
44. Scherf U, Ross DT, Waltham M, *et al*. A gene expression database for the molecular pharmacology of cancer. *Nature Genet* 2000, **24**, 236–244.
45. Esteller M, Garcia-Foncillas J, Andion E, *et al*. Inactivation of the DNA-repair gene MGMT and the clinical response to alkylating agents. *N Eng J Med* 2000, **343**, 1350–1354.
46. Offit K. Genetic prognostic markers for colorectal cancer. *N Eng J Med* 2000, **342**, 124–125.