Science, medicine, and the future

Colorectal cancer

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Colorectal cancer is the second commonest fatal malignancy in both sexes combined, after lung cancer. The incidence in both developed and developing countries has increased over the past few decades, and in Britain the population lifetime risk of developing large bowel malignancy is 1:25. The overall five year survival is around 40%, but survival can be as high as 70% after curative surgery. The morbidity associated with the disease and its treatment is considerable. Almost all patients require surgical resection, but palliation is the only realistic hope for the substantial proportion of patients who already have disseminated disease. Although the outlook is poor, considerable financial resources are expended in combatting the disease. The estimated annual cost of treating confirmed cases in Britain exceeds £200m (\$320m). This figure substantially underestimates the true cost as it does not include money spent on excluding cancer in patients with large bowel symptoms. In addition, the recent widespread introduction of adjuvant chemotherapy will increase costs dramatically.

This review highlights recent advances in colorectal cancer research that should result in tangible benefits to the patient in the near future. However, the pace of discovery is so rapid that applications of research strategies not yet dreamt of are likely to become reality over the coming decade.

Familial risk

Colorectal cancer is largely an environmental disease, but genetic susceptibility has an important role. Two highly penetrant autosomal dominant predisposition syndromes, hereditary non-polyposis colorectal cancer and familial adenomatous polyposis, together account for 2-10% of all cases of colorectal cancer. Genetic loci have also recently been mapped for Peutz-Jeghers syndrome and a form of juvenile polyposis. However, some familial aggregation is less well defined and may be multifactorial or polygenic in origin or perhaps due to low penetrance autosomal dominant mutations or even recessive gene defects. I will consider the characterised dominant gene disorders and loose familial aggregation separately since the focus of future developments is likely to differ.

Advances in our knowledge of genetically characterised disorders will soon overcome the practical and financial constraints of detecting heterogeneous mutations in large numbers of samples and make population

Possible future developments

- Identification of new genes for susceptibility and of modifier genes
- Population based genetic screening will become feasible and allow targeting of colonoscopy and chemoprevention at high risk subjects
- Identification of genetic alterations in tumours, peripheral blood, lymph nodes, and bone marrow that predict prognosis
- Development of drugs for treatment and prevention based on defined molecular and genetic targets—for example, cyclo-oxygenase 2 inhibitors, DNA topoisomerase inhibitors, and pharmacomodulation of APC and β catenin
- Use of gene therapy to target treatment at tumour cells, modulate immune response, and to introduce suicide genes

screening feasible. Population genetic screening for susceptibility to colorectal cancer will enable gene carriers to be identified before the onset of disease and offered intensive surveillance, enrolled in chemoprevention trials, or even offered prophylactic colectomy.

Major dominant gene disorders

Familial adenomatous polyposis

Familial adenomatous polyposis is responsible for probably less than 0.5% of all cases of colorectal cancer but tends to arise in people aged 20-30 years. The risk of cancer is so high that identification of gene carriers is essential. Genetic analysis can be done for every patient (new cases or established families) because a single gene (APC) is responsible for the disorder. In addition, the characteristic phenotype, lack of mimicking diseases (phenocopies), and relatively small number of patients affected have simplified the implementation of mutation analysis. The substantial clinical benefits of such genetic testing are already clear. Children from families known to be affected by familial adenomatous polyposis are now offered direct mutation testing at about 13-14 years old. This is much less invasive than the previous practice of sigmoidoscopy every six months until age 30 and then less frequently. Endoscopy can now be restricted to people from the few families where a mutation cannot be detected and those known to carry the mutation.

In addition to establishing the genetic diagnosis, mutation analysis can provide pointers to best clinical

practice.¹ Mutations in certain regions of APC tend to be associated with extracolonic symptoms such as desmoid tumours, while mutations in other regions correlate with severity of colonic disease. Patients with a genotype that suggests a high risk of cancer after colectomy and ileorectal anastomosis may therefore be best served by proctocolectomy and ileoanal pouch as the primary procedure. As correlation between genotype and phenotype improves, it is likely to become an important clinical parameter.

The effect of modifier genes on presentation of disease is also receiving considerable attention. Advances in whole genome analysis and informatics should make it possible to predict the natural course of disease by looking at data from the primary gene and perhaps two or three other contributing genes. Similarly, assessment of genotype will allow prediction of response to preventive treatments such as aspirin and other prostaglandin synthetase inhibitors.

A third application of molecular biology in familial adenomatous polyposis is in discovering how the APC gene works and the pathways involved. Such research also has relevance to sporadic cancer since most sporadic tumours have APC gene defects. It should allow the development of specific inhibitors to treat established polyposis in familial adenomatous polyposis or to prevent sporadic colorectal neoplasms in the general population.

Hereditary non-polyposis colorectal cancer

Hereditary non-polyposis colorectal cancer is an autosomal dominant genetic disorder characterised by the onset of colorectal cancer at an average age of 45 years and frequent occurrence of other cancers such as uterine, ovarian, gastric, and upper urinary tract. Four genes that participate in DNA mismatch repair have been shown to be responsible.² Although dominant inheritance is not as common as for familial adenomatous polyposis, smaller families with a few distantly related people affected are common (fig 1). Around 10% of people with colonic cancer have an affected first degree relative,^{3,4} and it is unclear how many of these might carry a mutation in a gene for non-polyposis colorectal cancer. Recognition of affected families is confounded by lack of pathognomonic features, deficiencies in family information, adoption, early death of relatives from unrelated causes, and incomplete gene penetrance. This means that an appreciable proportion of carriers of non-polyposis colorectal cancer genes with cancer are not recognised as such.

Preliminary data from affected families indicate that intensive colonoscopic screening reduces cancer mortality.⁵ Proactive population genetic screening for genes for hereditary non-polyposis colorectal cancer would prevent an estimated 1500 cancers a year in Britain. However, technical developments will be essential to achieve this. One is organisational, such that patients with early onset disease and the characteristic tumour genetic instability² can be identified as they are most likely to harbour mutations. The second development is technical progress in large scale analysis for mutations distributed throughout several genes. Microchip technology looks as though it could make this possible. Arrays of oligonucleotide combinations are set out on a chip analogous to a computer chip and can be designed to recognise random or specific sequences. The

Fig 1 Genealogical tree of family affected by hereditary non-polyposis colorectal cancer. All those affected carry the same pathogenic mutation, which results in truncation of the protein product, but several unaffected relatives carry the same mutation

technique has already been used to screen for the BRCA1 gene.⁶ Giving blood tests to relatives of cancer patients with a defined pathogenic mutation will allow targeting of invasive colonoscopy to those at highest risk. In addition, randomised trials of new chemopreventive drugs will be possible by recruiting relative small numbers of gene carriers who have a high risk of colorectal cancer.

The inevitable technical advances that will occur in population mutation testing for susceptibility to colorectal cancer must be supported by high quality and accessible genetic counselling. People shown to be predisposed to cancer will need information and advice, which will require a large increase in the provision of trained genetic counsellors. Establishment of validated referral protocols and indications for recruitment to invasive colonoscopic screening will also be essential. Already large numbers of people who may not be at particularly high risk are being screened on the basis of their family history.⁷

Unmapped genetic susceptibility loci and modifying genes

Attempts to identify other genes that contribute to risk of colorectal cancer are already under way. The potential impact is substantial since most colorectal cancer falls into this category. As more genetic markers become available, automated scans of the whole genetic code have an increased chance of identifying the target locus. Once a genetic locus is identified, all sequenced genes in that area can be analysed for mutations and efforts made to detect the expressed genes mapping to that region. Advances in automation and bioinformatics will also allow assessment of the complex interactions between genetic and environmental influences.

The next phase of research in genetic susceptibility to colorectal cancer will undoubtedly be the unravelling of the complexities of multifactorial aetiology in the genetic susceptibility cancers (fig 2). Identification of modifying genes will increase the scope for pharmacological and genetic phenotype manipulation to slow or delay onset of disease. As an example, a locus that modifies the expression of an APC gene mutation in mice has already been identified, although there is no evidence that the related gene has any effect in humans.

Fig 2 Schematic representation of genetic and environmental interactions relevant to colorectal cancer. Studies of mutational spectra indicate that specific mutagens in faeces are not involved in inducing somatic mutations in known cancer genes

Somatic cell genetics

Somatic cell genetics refers to the molecular events that occur in cells during neoplastic transformation. Information on the genetic basis of colorectal cancer is the most complete of any of the common solid tumours, partly because of the ready availability of normal mucosa and benign and malignant neoplasms. Genes implicated at various stages of neoplastic progression have been identified, and doubtless more will be detected. The roles of APC, K-ras, DCC, DPC4, p53, TGF β , RII, and DNA mismatch repair genes are well reviewed,²⁻⁸ but knowledge of somatic cell genetics will also greatly affect the assessment of prognosis of sporadic cancer and development of new treatments.

Molecular genetic analysis in diagnostic and prognosis

Although most genetic alterations detected in primary tumours are not present in normal tissues, some changes can indicate constitutional gene defects. The use of specific antibodies that recognise altered proteins resulting from pathogenic mutations will allow routine molecular assessment of tumours. Such antibody technology will allow detection of people likely to carry susceptibility genes and has already been used to detect inactivating mutations in DNA mismatch repair genes.⁹

Genetic alterations in the primary tumour have been shown to reflect tumour progression. For example, the DCC gene on chromosome 18q tends to be inactivated in large villous adenomas but not in small polyps.²⁻⁸ This is mirrored by the demonstration that DCC inactivation correlates with poor prognosis independently of lymph node status.¹⁰ Elucidation of the role of other genes in progression to invasive malignancy will greatly increase the array of prognostic markers available to pathologists.

Genetic analysis also has considerable potential in detecting tumour specific genes outside the primary lesion. Circulating tumour cells are well described in

colorectal cancer, but detection is difficult. Sensitive, automated genetic techniques are being developed to detect APC, p53 and K-ras mutations in circulating blood, lymph nodes, and bone marrow. Studies have already shown that genetic analysis to detect p53 or K-ras mutations in lymph nodes is more sensitive than conventional pathological analysis¹¹ and that tumour specific cytokeratin 18 antigen in bone marrow has prognostic value independent of standard pathological staging.¹² Tumour derived DNA with K-ras mutations has also been detected in plasma in a high proportion of patients.¹³ Molecular approaches to detecting minimal residual tumour deposits are more sensitive than traditional pathological assessment and have potential for automation.

New treatment and prevention targets

As genes are identified and their interactions become unravelled, the scope for biological modification increases exponentially. General examples of such rational drug design for colorectal cancer include DNA topoisomerase inhibitors such as CPT11 (Irinotecan) and thymidylate synthase inhibitors such as AG337 (Thymitaq), which are already in clinical trials. Development of specific drugs to counteract disruptions of cellular pathways that are exploited by cancer will reap huge rewards. The pivotal role of APC as an initiator of colorectal neoplasia in both sporadic colorectal cancer and familial adenomatous polyposis exemplifies the relevance of this approach.

Several studies have shown that non-steroidal anti-inflammatory drugs reduce polyp counts in familial adenomatous polyposis and are associated with reduced incidence and mortality from sporadic colorectal cancer.¹⁴ Findings in transgenic animals indicate that reduction in polyp counts is due to inhibition of cyclo-oxygenase 2 and that drugs designed to inhibit this enzyme are more effective than aspirin-like drugs, which inhibit cyclo-oxygenase 1 and 2.¹⁵ Other studies have shown that APC binds to β catenin in competition with a transcription activator (tcf) that is functionally active only when bound to β catenin.¹⁶ Hence elucidation of pathways upstream and downstream of APC will allow rational development of drugs to treat established disease and, perhaps most exciting of all, for chemoprevention.

Immunotherapy will also improve with increased understanding of gene expression. Passive immunotherapy with monoclonal antibodies to the antigen 17-1A has already been shown to confer survival benefit in colorectal cancer when used as an adjuvant therapy.¹⁷ Laboratory studies and clinical trials are also under way to investigate active immunisation against a related antigen (GA733) by using gene therapy. The scope for immunotherapy will be expanded enormously as more biological targets are identified by molecular biological research. Recent advances in antibody technology will also have a key role. Antibodies can now be generated that recognise specific human antigens, but the portion that binds immunologically potent cells is "humanised" to provide maximal biological effect in vivo.

Gene therapy

The introduction of exogenous genes to combat colorectal neoplasia holds enormous potential, although considerable practical difficulties remain. Corrective gene therapy for inheritable syndromes such as familial adenomatous polyposis and hereditary non-polyposis colorectal cancer is a formidable challenge since transgene expression would have to be maintained over decades. Hence replacement gene therapy in hereditary non-polyposis colorectal cancer is improbable. Gene therapy may have a place in familial adenomatous polyposis and could be targeted at treatment of the retained rectum after colectomy with ileorectal anastomosis or treatment of extensive upper gastrointestinal neoplasia.

Colorectal cancer is a multistep disease and so treatment of established tumours by replacing single genes is unlikely to be effective. Nevertheless, replacement of p53 or APC genes has shown considerable antitumour activity in vitro and in animal studies. Replacement of p53 may also have value in sensitising cancer cells to conventional treatments.

To maximise tumour damage and minimise damage to host tissues, fragments of regulatory sequences (promoter) can be engineered to drive tumour specific transgene expression. This approach is already being assessed in clinical trials to target expression of suicide genes in colorectal cancer.¹⁸ Recognition sequences for oncoproteins which are aberrant in cancer cells such as p53 have also been introduced to drive transcription of suicide genes.¹⁹ Another elegant example of exploiting tumour biology to target gene therapy involves the use of recombinant adenovirus that replicates selectively in cells with mutant p53.²⁰ Considerable antitumour effect has been shown in laboratory studies, and clinical trials are under way.

Advances in the understanding of gene expression will also increase the arsenal of potential effector genes. Currently two main categories are being explored in colorectal cancer: immune modulators and suicide genes. Gene therapy allows delivery of a locally high cytokine concentration to minimise side effects. Treatment with interleukin 2 and interleukin 12 has proved particularly effective in experimental studies of colorectal liver metastases.²¹ Cancer cells escape immune surveillance, partly because they do not express costimulatory molecules such as the B7 family. B7 gene therapy is being tried to confer some of the properties of the antigen presenting cell on the tumour. Vaccination with DNA for specific tumour antigens such as GA733 also holds considerable potential.

Several suicide gene approaches are currently being evaluated. These aim to exploit the conversion of a non-toxic prodrug to a cytotoxic metabolite and include cytosine deaminase, which converts 5-fluorocytosine to the active 5-fluorouracil, and herpes simplex virus thymidine kinase, which converts ganciclovir to the active triphosphate form. Suicide genes have been shown in laboratory studies to work synergistically with immune modulators.

Conclusions

The rapid progress in understanding of the function of cloned genes and the frequency of advances that open

whole new research avenues make it difficult to predict how biomedical research will translate to treatment of colorectal cancer. Primary prevention will soon be possible by identification of individuals carrying mutations in susceptibility genes and by development of new chemopreventive drugs designed on the basis of knowledge of the biological pathways involved. It also seems likely that molecular genetic research will provide prognostic tools and new biological and pharmacological treatments.

The human genome project will soon have completed mapping and sequencing of all human genes. The next challenge will be to determine which genes influence cancer susceptibility and to dissect complex cellular pathways to identify new approaches to treatment. The so called "new biology" is not a flash in the pan—it is the exponential accrual of biological knowledge on a scale that has never before been possible. The application of that knowledge is all that is required to combat a disease that has thus far thwarted our best efforts.

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