Molecular biology of colorectal neoplasia

Large bowel cancer is a major cause of morbidity and mortality in the western world. In England and Wales approximately 8500 men and 9000 women die of the disease every year.1 Yet despite this, relatively little is known of its pathogenesis in the vast majority of sporadic cases. In 1954, Armitage and Doll proposed that common cancers arise as a result of the accumulation of as many as seven events.2 Most scientists now believe that the molecular substrate for these critical events in tumorigenesis are genes that regulate cell proliferation and differentiation – that is, oncogenes and tumour suppressor genes. Alterations to the expression or structure of these genes may result in the perturbation of cell growth, which is the hallmark of neoplasia.

The past 10 years has seen an explosion in our knowledge of the molecular biology of cancer, and nowhere has this been as exciting as in the field of large bowel cancer. Research into colorectal tumours has directly or indirectly contributed to the discovery of four new tumour suppressor genes; APC, DCC, and p53, the latter probably representing the commonest genetic abnormality so far described in human cancer.3 Therefore, despite being unclear about the environmental agents that promote genetic changes in the large bowel, in few other tumour systems are we as close to identifying the critical events which underlie malignant, neoplastic behaviour as we are in colorectal cancer.

Oncogenes

Originally described as retroviral genes responsible for in vitro cell transformation and the development of certain animal tumours (v-onc), homologs have subsequently been identified in normal human cells (proto-oncogenes) and, in activated form, in human tumours (v-onc). Many have a physiological role in the regulation of cell division and differentiation. It is not surprising therefore that they are a common target for mutagenesis in neoplasia.

Most frequently altered in large bowel tumours are c-Ki-ras and c-myc. c-Ki-ras, one of the family of ras oncogenes, encodes a 21kD G-protein involved in the transduction of mitogenic signals across the cell membrane.4 Point mutations in codons 12, 13, or 61 have been identified in 39 to 71% of large bowel cancers, as well as 42% of adenomas.5-7 This results in the inability of the protein to hydrolyse bound GTP to GDP, and since GTP-ras is believed to be the active form of the protein this may deliver a continual signal to the cell to divide. Apart from point mutation, another way in which oncogenes can be activated is by over expression. This seems to be the mechanism for c-myc activation in colorectal tumours. C-myc encodes a nuclear phosphoprotein that is induced during cell proliferation, and is probably necessary for DNA synthesis.8 Several groups have reported raised c-myc RNA values in 60-70% of carcinomas.9,10 Similar levels have also been described in adenomas. While in some cases this may merely reflect the larger growth fraction found in tumours compared with normal mucosa, genuine over expression does seem to occur in a proportion of cases.11 Amplification of c-myc has been described in a minority (7%) of tumours but correlates poorly with RNA content.12,13 The reason for deregulated expression of the gene is therefore presently unknown.

Other oncogenes less frequently altered in large bowel tumours include c-src, raised in 62% of cancers; c-myb, deleted in 9%, and c-erbB-2, amplified in 4%.14-16

Tumour suppressor genes

Numerically a much smaller group, tumour suppressor genes inhibit cell proliferation and tumorigenicity. Whereas oncogenes classically act in a transdominant fashion – that is alteration to one allele is sufficient to cause transformation, suppressor genes are recessive. Loss of tumour suppressor function requires inactivation of both alleles, usually by chromosomal deletion or point mutation, or both.

Tumour suppressor genes seem to be very important in colorectal carcinogenesis. The hereditary condition, familial adenomatous polyposis (FAP), which confers susceptibility to colonic cancer, is determined by point mutations in a tumour suppressor gene, APC, inherited through the germ line. Localised to chromosome 5q.21 by Bodmer et al in the United Kingdom, and Leppert et al in the United States, the candidate gene encodes a large 2843 amino acid protein, whose function is, as yet, poorly understood.16-18 While the inherited allele is mutated, the normal allele is subsequently deleted in a somatic, mitotic event involving variable sized fragments of the long arm of chromosome 5. This acquired loss of heterozygosity for chromosome 5q can be detected using restriction fragment length polymorphism (RFLP) markers in 36% of sporadic cancers, as well as FAP carcinomas.19,20 In a survey of 79 unrelated FAP patients, mutations were found in the APC gene in 67%.21 More than two thirds of mutations were located in the 5' half of the last exon, and over 90% resulted in truncation of the APC gene product. Miyoshi has described similar mutations in sporadic adenomas and carcinomas.22 Inactivation of the APC gene therefore seems to play an important role in sporadic colorectal tumorigenesis as well as in FAP.
Interestingly, a murine counterpart of human FAP exists. Mice expressing the Min trait (multiple intestinal neoplasia) develop large numbers of adenomas in the small and large bowel and are susceptible to gastrointestinal cancer. The trait is transmitted as an autosomal dominant, and linkage analysis has shown cosegregation with a nonsense mutation in the murine homolog of the APC gene.

During the exploration of the long arm of chromosome 5 for the APC locus, deletions and point mutations were identified in a second large gene named MCC (mutated in colorectal cancer). Up to 55% of cancers have subsequently been found to contain deletions involving MCC. Although now believed not to be the gene responsible for FAP, MCC still clearly represents a common site for mutagenesis, and may therefore be another colorectal tumour suppressor gene. One intriguing possibility is that the proteins encoded by MCC and APC interact to form a biologically active complex. Mutation of either gene would then be sufficient to inactivate the complex.

DCC (deleted in colorectal cancer) is a candidate tumour suppressor gene located on chromosome 18. Deletions affecting the DCC locus have been found in 73% of cancers, but only 11% of adenomas. Parallel studies of expression of the DCC gene confirm that while mRNA transcripts are present in normal colonic mucosa and adenomas, the gene is frequently unexpressed in carcinomas. Preliminary characterisation suggests that the gene product resembles a cell adhesion molecule and may therefore regulate the interaction of a cell with its environment. When DCC was first localised to chromosome 18q, it was suggested that it may determine HNPC (hereditary non-polyposis colorectal cancer) in the same way that APC determines FAP, since HNPC shows linkage to chromosome 18 markers. This has not, however, proved to be the case.

In 1989 Baker et al showed that deletions of the short arm of chromosome 17 were associated with point mutations in the p53 allele on the homologous chromosome. Since then much evidence has accrued that p53 is a tumour suppressor gene involved in a wide range of human malignancies. Deletions of chromosome 17p are found in 75% of colorectal cancers and are highly correlated with mutation of the remaining p53 gene. Ninety five per cent of large bowel cancers showing loss of heterozygosity for 17p alleles also contain a point mutation. A similar association has been described for adenomas where deletion is much less common. Many of the mutations in colorectal cancer are of a dominant-negative type – that is, not only do they result in loss of tumour suppressor activity, they also confer the ability to transform NIH/3T3 cells. This is probably achieved by mutant p53 binding wild type p53 protein and sequestering it in a biologically inactive oligomer. Consequently, unlike other tumour suppressor genes, only one allele need be mutated to produce a phenotypic effect.

Mutation of the gene frequently results in stabilisation of the normally short lived protein. Immunocytochemistry can then be used as a marker of p53 mutation, although DNA sequencing remains the gold standard. Using immunohistochemistry, over expression of nuclear p53 protein has been found in 42% to 67% of carcinomas compared with 8% of adenomas and 0% of normal mucosa.

The presence of other chromosomal deletions in colorectal cancer suggests that several more tumour suppressor genes have yet to be identified. Of particular interest is the frequent loss of alleles from chromosomes 1p, 8q, 13q, and 22q.

Adenoma-carcinoma sequence
Comparison of the frequency with which tumour suppressor and oncogenes are altered in adenomas and carcinomas suggests that there is a preferred order for their occurrence in the adenoma-carcinoma sequence (figure). For instance, while deletion of DCC on chromosome 18q is present in 75% of cancers, only 11% of adenomas show allelic loss. A similar trend is seen for p53 gene mutation, whereas mutations of APC, MCC, and c-ras are equally common in premalignant and malignant tumours. Exceptions to the rule clearly exist, however, and Fearon has emphasised that it is the accumulation and not the order of these changes which is important. Nevertheless, the differences in prevalence of p53 mutation and 17p and 18q deletions between premalignant adenomas and adenocarcinomas is striking, and cannot easily be dismissed. It may be that p53 or DCC inactivation have no effect until other constraints on cell behaviour are removed – for example by APC inactivation, and that in their absence alterations to p53/DCC are not selected for.

Alternatively, changes to p53 and DCC may be much more closely related to malignant behaviour than c-ki-ras or c-myc activation.

Changes to other genes may be implicated in metastasis, which depends upon the ability of a tumour cell to invade blood vessels, survive the host immune response, and grow in a foreign microenvironment. Key determinants of metastatic behaviour include production of growth and tumour angiogenesis factors; modulation of cell surface receptors and adhesion molecules; secretion of enzymes such as collagenase and plasminogen activator, and changes in the expression of MHC antigens. Pignatelli et al have, in particular, highlighted the importance of ECM (extracellular matrix) receptors to tumour behaviour.

In 1988, Steeg et al identified a gene, nm23, whose expression correlates strongly with the ability to metastasise. Deletion of nm23 may be a critical event in the

A model for the molecular basis of the adenoma-carcinoma sequence.
lymphatic and haematogenous dissemination of carcinomas.

Consideration of the genetic lesions in any one tumour makes it apparent that carcinogenesis is a heterogenous process. Non-oncogene or tumour suppressor gene has yet been implicated in all large bowel cancers. In a study of some of the commonest abnormalities found in colorectal cancer, Vogelstein found that while over 90% of carcinomas contained two or more alterations, a minority, constituting less than 10% of the total, contained none. Which genes are involved in the malignant conversion of this unusual subgroup of tumours presently remains a mystery.

In vitro certain combinations of oncogenes cooperate to transform primary rat cells. One of these combinations is p53 and c-ras, both frequently activated in large bowel cancers. It might be anticipated that tumour cells possessing a mutant p53 and a mutant K-ras allele would have a growth advantage and be selected for during in vivo tumorigenesis. This does not seem to be the case, however. In a series of 100 colorectal cancers analysed for K-ras mutation and p53 over expression, the proportion of tumours showing both abnormalities was only that to be expected by chance.43

Adenoma-carcinoma sequence: an in vitro model

Recent work in which an adenoma cell line was exposed to chemical carcinogens has duplicated many of the in vivo genetic findings. Adenoma cells converted to anchorage independent growth and tumorigenicity by MNNG (N-methyl-N-nitro-N-nitrosoguanidine), display aneuploidy, allelic loss on chromosome 1p, and deletion of chromosome 18. The same cells also become less responsive to the growth inhibitory effect of transforming growth factor beta (TGFβ). 47

Ulcerative colitis

Several studies have now shown identical genetic lesions in ulcerative colitis associated dysplasias and cancer to those found in sporadic adenomas and carcinomas. Bell et al described mutations of c-Ki-ras in 24% of ulcerative colitis associated cancers, while Greenwald et al reported ras mutations, p53 gene deletions, and MCC/APC deletions in both carcinomas and premalignant dysplasia. 48

Clinical implications

What are the implications for clinicians of the wealth of information now available? The impact of molecular biology will eventually revolutionise our whole way of thinking about cancer and its management, but for the time being three areas are receiving particular attention:

SCREENING

Screening for FAP in affected families presently involves annual endoscopic examination starting from 14 years of age. This inevitably results in unnecessary inconvenience and distress for those not carrying the gene, not to mention the expense of regular colonoscopies. Use of microsatellite probes mapping within APC, and eventually gene sequencing, will permit early, possibly even prenatal diagnosis of FAP. This will save time and money and enable a more economic allocation of clinical resources. Similar strategies may eventually also allow screening for HNPCC, peutz-jeghers, and juvenile polyposis once the responsible genes have been identified.

Can molecular biology help in the screening of other high risk groups? Almost certainly, yes! Detection of genetic alterations in ulcerative colitis associated carcinomas and dysplasia provides a range of potential markers for early diagnosis. Sidransky et al have recently screened the stools of nine patients with large bowel tumours containing mutant K-ras alleles, and identified mutant DNA in eight faecal samples. The survey of exfoliated cells in stool samples for mutated DNA or tumour products, or both, should theoretically provide an alternative approach to periodic colonoscopy which would be more acceptable to both patient and clinician. At present, however, screening for c-ras mutations on their own is too insensitive to provide any more than an auxiliary technique to colonoscopy, since mutant alleles are found in only 24% of carcinomas, and between 0% and 33% of high grade dysplasia. 45, 46 Nevertheless, the identification of more common alterations such as p53 mutation or screening for a panel of genetic lesions, or both, may in future make genetic screening of stool samples a viable proposition.

While faecal occult blood testing is likely to prove the most cost effective method of screening large populations, genetic tests could initially be targeted at selected high risk groups. This would include patients who had undergone previous surgery for large bowel cancer and individuals with a strong family history of colorectal neoplasia, as well as those with chronic ulcerative colitis.

PROGNOSIS

In spite of the vast array of clinical and pathological features which help predict patient outcome, individual tumour behaviour can still not be accurately determined for the majority of patients who fall into Dukes's stage B and C1, with 77% and 41% 5 year survival respectively. Radiological investigation of the liver by ultrasound, computed tomography, or magnetic resonance imaging can help identify individuals with small hepatic metastases while pathological examination of the circumferential resection margin may predict local recurrence of rectal cancers. Nevertheless, more subtle prognostic indicators are urgently needed to assess a tumour's capacity for metastatic spread, and to plan adjuvant radio- or chemotherapy. A special example of this is the small, mobile cancer in the rectum which is amenable to local excision. Up to 12% of these tumours still confined to the bowel wall have already spread to regional lymph nodes. A new way of predicting lymphatic metastasis is needed to avoid undertreatment of these patients.

Analysis of oncogenes and tumour suppressor genes may provide a better guide to tumour behaviour than conventional techniques. Two large studies have shown genetic lesions, including chromosome 17p, 18q, and 1p deletion, to be prognostic indicators. 45, 46 In multivariate analysis, chromosome 17p deletion proved to be independent of Dukes's stage or grade. In vitro, oncogenes cooperate to transform cells and render them tumorigenic. It is possible that in the future combinations of oncogene and tumour suppressor gene alterations may yield a unique insight to the invasive or metastatic capability of a tumour. For instance we have recently shown that colorectal cancers that contain c-Ki-ras mutations and over express p53 have a far worse prognosis than those showing either alteration on its own. 45

Nm23 is one possible example of an anti-metastasis gene. Deletion of the gene in breast cancer is associated with reduced patient survival and increased risk of lymph node metastasis. Recently Cohn et al found that 73% of 11 large bowel cancer patients whose tumours had lost the nm23 allele developed metastatic liver disease compared with only 20% of the remainder. Inactivation of nm23 may therefore prove a useful guide to the risk of distant metastasis.

THERAPY

Very much in the future, but potentially the most exciting
aspect of cancer biology, is the prospect of gene therapy. Supplementation of tumour suppressor genes or the use of specific oncoprotein antagonists may inhibit tumour cell growth in vivo in the same way that they do in vitro. Recent work suggests that correction of one tumour suppressor gene defect may inhibit tumorigenesis, even in the presence of several other abnormalities. This would greatly facilitate the treatment of genetically complex tumours like colorectal cancer. Obviously, there are still many other problems to be overcome, including the possible effect of targeting genes and proteins which normal cells depend on for their own growth control. However, the challenge is there to be met and oncogene manipulation may provide the next real step forward in a disease which has proved largely refractory to radio- or chemotherapy, and in which prognosis has changed little over the past 40 years.

We should like to thank Miss J J Hamblin and Mr C A R Hay for their help in the preparation of this manuscript.

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