Leading article

Oncogenes and gastrointestinal cancer

Even after overcoming prejudice against basic science, restrictions on research funding and an uncertain career structure, the persistent clinical scientist still wanting to develop an interest in the applications of molecular cell biology to medicine will face one further hurdle: the sheer density of much of the scientific writing in molecular biology, which has restricted its readership within the medical world. It was Bertrand Russell who, finding that the philosophy of Hegel was uniquely obscure, concluded that it did not amount to anything of significance. There seems to be a danger that many clinicians and possibly also some clinical scientists, particularly in this country, may reach an erroneous conclusion about the relevance of much of what is going on in molecular biology and for the same reason. Of course, Russell’s writings are models of clarity and wit, sufficiently so for him to have been awarded the Nobel prize for literature. The molecular biologists of today have been picking up most of the recent Nobel prizes for medicine and indeed look set to continue to do so for many years to come. But what is likely to be the richest prize of all? We do not have to search very hard for the answer. It is certain to be the unravelling of the molecular pathology of cancer. We are not close to this goal but such is the pace of scientific progress that the more optimistic amongst us feel that it might just be achieved before the close of this century. If it is, then perhaps the seminal observation along the road to a fundamental understanding of carcinogenesis will be recognised to have been the discovery of cellular oncogenes. Why are oncogenes likely to be so important and what, in particular, is their relevance to gastrointestinal cancer?

The oncogene story begins in 1911, when Rous discovered that a virus could cause a transmissible tumour in chickens.1 Such RNA tumour viruses, or retroviruses as they are now known, are genetically simple structures. The viral genes responsible for neoplastic transformation have been readily identified, and they have come to be known as oncogenes (or, v-onc). The next key step was the quite extraordinary finding that cells uninfected with retrovirus contain DNA sequences homologous with viral oncogenes.2 Not only was this true for the Rous sarcoma virus oncogene (src)* and uninfected chicken cells, but it held true also for other viral oncogenes and many vertebrates (including man).3 These cellular homologues of viral oncogenes are known as cellular proto-oncogenes (c-onc) and have been now recognised in all vertebrates. Indeed, they are highly conserved throughout evolution, which suggests that the protein products of these genes play an important role in the cell. There are known to be more than 40 oncogenes and proto-oncogenes and we have a broad idea of the range of function of their protein products, which seem to fall into three categories:

* Viral and cellular oncogenes are given three-letter names which are abbreviations derived from their respective tumour viruses: for example, myc from avian myelocytomatosis virus, ras from rat sarcoma virus.
1 Protein kinases
The function of these proteins is not well understood but they are located on the plasma membrane. Much interest has centred around the link between growth factors and oncogenes, particularly as growth factor receptors have protein kinase activity. For example, the protein encoded by the c-sis oncogene is closely related structurally to the beta subunit of platelet-derived growth factor.15

2 Guanosine triphosphate (GTP)-binding proteins
Guanosine triphosphate seems to be involved in sending signals from the cell surface to its interior, and oncogenes of the ras family can bind GTP.6

3 Nuclear binding proteins
A third group of proteins is associated not with the plasma membrane but with the cell nucleus. The c-myc oncogene product appears to be involved in replication of DNA18 and the fos gene product may influence the genesis of messenger RNA.4

It seems then that proto-oncogene products are not just important, but may well be crucial in the control of normal cell function. But what is the evidence that abnormalities in the expression of proto-oncogenes actually leads them to function as oncogenes and hence to cause cancer in man? In 1982, it was reported that an extract of DNA from a human bladder cancer could produce neoplastic transformation of cells in tissue culture, and furthermore the sequence of tumour DNA responsible for this effect was a homologue of the ras oncogene.10,11 Subsequently it has been shown that an alteration in a single nucleotide base (point mutation) within the ras gene leads to a single amino acid substitution in the ras protein which, in turn, alters the function of this peptide sufficiently for neoplastic transformation to result.12 Other genetic mechanisms that appear to be involved in activating proto-oncogenes include translocation – for example, in chronic myeloid leukaemia in which the abl oncogene is translocated from chromosome 9 to chromosome 2213 – and amplification (as in neuroblastoma, in which multiple copies of the N-myc gene occur as a result of gene duplication).14

The potential role of abnormalities in oncogene expression in the development of gastrointestinal cancer has been intensively studied in recent years. This is, in part, because of the enormous importance of such tumours as a cause of morbidity and mortality. There are, however, two other special reasons why gastrointestinal tumours have attracted the attention of molecular oncologists. Not only is the gastrointestinal tract a readily available source of fresh tumour tissue but, particularly in the colon, there are a number of relatively common and well-defined premalignant disorders. These include not only familial adenomatous polyposis which, unless treated, leads to the inevitable development of colon cancer, but also sporadic adenomatous polyps and longstanding ulcerative colitis in which the risk of malignancy is appreciable. Studies which compare levels of oncogene expression in normal, premalignant and frankly neoplastic tissue specimens afford the opportunity of finding out whether proto-oncogenes may indeed by causally implicated in carcinogenesis.

Much of the early data on oncogene expression in the gut came from work
Oncogenes and gastrointestinal cancer

on tumour cell lines. These results are of interest but may not necessarily be applicable to human tumour biology. Let us examine what has been found when colorectal tissues have been studied directly.

Slamon et al found increased expression of *fos*, *myc* and *ras* oncogenes in a majority of colorectal carcinomas, but there was no enhancement of the expression of 11 other oncogenes. Of 29 colorectal adenocarcinomas studied by Erisman et al, 72% had considerably raised levels of expression of *c-myc*. A further study reported that levels of *myc* expression were significantly greater in tumours in the left colon compared with right sided lesions, but the significance of this finding is not clear. It seems then that enhanced expression of the *c-myc* gene is a common finding in colon cancer, but there does not appear to be a consistent associated abnormality within the gene. Despite the fact that in the study of Erisman et al most tumours did show enhanced expression of *c-myc*, neither gene amplification nor rearrangement were seen in any case. Alexander et al reported amplification of *c-myc* in two of nine fresh colorectal tumours, while Yokota et al also found *myc* amplification in two of 32 colon cancers.

Some encouragement for oncogene enthusiasts, however, comes from recent studies in which the molecular genetics of the *ras* gene have been studied. Meltzer and colleagues found mutations of *myb* or *ras* genes in 19% of colon cancers, but their presence did not correlate with Dukes’ stage, tumour progression or patient’s survival. Bos and coworkers reported that 40% of colorectal cancers showed mutation of the *ras* gene. Furthermore, *ras* mutations were seen in the adenomatous and carcinomatous regions from five of the six tumours in which benign and malignant tumours coincided. Forrester et al noted *ras* mutations in the same proportion of colorectal cancers, and additionally in seven of eight villous adenomas. These exciting results raise the possibility that activated *ras* genes contribute to the early stages of colorectal tumour genesis in man.

Another approach to the study of oncogene expression has been to investigate the expression of oncogene protein products. Raised amounts of the *ras* oncogene product (p21) were detected in nine of 17 colorectal carcinomas, with the most deeply invasive tumours showing normal p21 concentrations, but in contrast, metastases had lower levels. Immuno-histological studies of p21 in sections of benign and malignant colorectal tumours revealed that increased p21 concentrations were present only in the adenomas. These data are also consistent with the hypothesis that *ras* expression is a contributory factor in early tumour formation. A uniform pattern of results from studies of p21 has, however, yet to emerge.

The development of monoclonal antibodies to the protein product of *c-myc* (p62*^c-myc*) has allowed the distribution of the protein to be determined by immunohistology. A flow cytometric assay has also been developed, which allows quantification of the protein in cell nuclei derived from archival pathology material. By comparison with normal colon and colorectal carcinomas, sporadic adenomatous polyps had the most intense staining of p62*^c-myc*, particularly in areas of dysplastic change. p62*^c-myc* is a nuclear associated protein, but exclusive cytoplasmic staining at the epithelial surface was characteristic of normal colorectal biopsies. By contrast, adenomatous and malignant polyps from patients with familial adenomatous polyposis and biopsies of high grade epithelial dysplasia and carcinoma from patients with ulcerative colitis showed predominant...
nuclear staining at the mucosal surface. The consistent but apparently paradoxacl finding of the c-myc protein product in the cell cytoplasm of normal colonic epithelium rather than in the nucleus (as one might expect) has not been explained, but it does not seem likely to be an artefact of the immunohistological processing. Using the technique of flow cytometry, in comparison with the findings in normal colon, markedly increased concentrations of nuclear p62c-myc were detected in high grade epithelial dysplasia and frank neoplasia in ulcerative colitis.33

Carcinogenesis is a multistep process and the events leading to the formation of colorectal cancer (or any other solid tumour) are unlikely to be the result of the action of a single oncogene. Nevertheless, we have seen that over a period of just a few years some intriguing results have emerged. It now appears that ras proto-oncogene mutation may be an important step in initiating formation of colonic tumours. The data from the studies on myc are somewhat less clear. Although the precise function of the c-myc gene has not been established, it may have a role in regulating the cell cycle.34 If this is correct, abnormalities of myc expression may be the result, rather than the cause of disordered cell growth.35

The sheer pace with which one discovery follows upon another is electrifying. It has to be said that clinical applications have yet to appear, but it seems beyond doubt that in the near future we will see the fruits of this research being applied not only to diagnosis, but also to treatment of malignant disease. One can only express the hope that British scientists and clinicians will be in a position to participate fully.

IAN FORGACS

Gastrointestinal Unit,
Dulwich and King’s College Hospitals,
London SE5 9RS

Address for correspondence to: Dr Ian Forgacs, King’s College Hospital, Denmark Hill, London SE5 9RS.

References
2 Stehelin D, Varmus HE, Bishop JM, Vogt PK. DNA related to the transforming gene(s) of the avian sarcoma viruses is present in normal avian DNA. Nature 1976; 260: 170–3.
Oncogenes and gastrointestinal cancer


11 Der CJ, Krontiris TG, Cooper GM. Transforming genes of human bladder and lung carcinoma cell lines are homologous to the ras genes of Harvey and Kirsten sarcoma viruses. *Proc Natl Acad Sci USA* 1982; 79: 3637–40.


