The somatic evolution of cancer

The Harveian Oration of 1996

Sir Walter Bodmer
MA, PhD, FRCPath, FRS

The Harveian Oration is given annually at the College under an indenture of William Harvey in 1656. The 1996 Oration was given on 17 October 1996 by **Sir Walter Bodmer**, Principal of Hertford College, Oxford. The full text is available from the publications department at £3.00 per copy

We owe the first step in our understanding of cancer to Rudolph Virchow, the eminent pathologist of the 19th century, who, following the enunciation of the cell theory by Schwann in the 1830s, described in his great pathology book of 1858 [1] how cancer was a disease of cells. He coined the term 'leukaemia', meaning white blood, for cancer of the white cells of the blood.

The next major advance in understanding came from Boveri's hypothesis, put forward in 1914, that cancer was associated with abnormalities of the chromosomes [2], and from Tyzzer and Strong's experiments on transplantable tumours in 1916 [3]. These established the notion of a somatic evolutionary basis for cancer.

The major theme of this paper is to show how the new genetics, working with cells and DNA, firmly establishes the somatic evolutionary basis for cancer; to illustrate this with a few examples particularly from work in my own laboratory over the last 15 or so years; and to emphasise some of the implications of this understanding for future approaches to the prevention, early detection and treatment of cancer.

Genetic changes in cancer

The first clear-cut evidence for a specific chromosomal change in a cancer was the discovery in the early 1960s by Noel and Hungerford of the Philadelphia chromosome in chronic myelogenous leukaemia (CML) [4]. This was interpreted by Janet Rowley some ten years later, after the development of chromosome banding techniques, as a specific exchange of material, namely a translocation, between chromosomes 9 and 22 [5]. This posed the question as to what was the nature of the specific genetic change at the breakpoints, and how this influenced the development of CML.

The interpretation of the Philadelphia chromosome translocation depended on the discovery by Varmus and Bishop and others of the dominant oncogenes carried by the oncogenic retroviruses, first identified by Peyton Rous in 1910 (see Franks and Teich [6] for general background). This was one of the first and most fundamental contributions of molecular biology to understanding cancer, since it was through this technology that it was possible to identify the specific and extra sequences carried by the retroviruses that enabled them to transform, and to show that these extra sequences were altered versions of normal genes present in normal host cells. Their dominance is established by the fact that these altered genes can carry out their transforming function in the presence of the normal, unaltered version of the corresponding gene. It was soon shown by a variety of approaches that these dominant oncogenes could be mutated in cancer by conventional genetic mechanisms that were not in any way connected with a virus. Subsequently, the work of Waterfield and others provided the first clues to the functions of the dominant oncogenes through showing that they coded for variant growth factors or their receptors, enabling them to function in an 'autocrine' manner without depending on the production of factors by another cell. This provided a readily understandable basis for the way in which a somatic mutation could give growth autonomy to a cell, allowing its clonal development and growth to be independent of the surrounding tissue, a cardinal feature of a tumour. Subsequent work showed that dominant oncogenes could also code for other key cellular functions involved in transmitting the signals from the growth factor receptor at the surface of a cell to the nucleus, and so providing the instructions for cell division to proceed and, for example, to override the processes of cell differentiation.

A simple and fundamental idea was suggested by Knudson in 1971, which forms the basis for the search for a totally different class of genetic changes in cancers that are fundamentally recessive in their mode of action [7]. He pointed out that, if a cancer arises through a series of somatic genetic changes, then sometimes one of those genetic mutations may be inherited through the germline and so be present in every cell in the body. Such individuals, therefore, have cells that are already one step along the somatic evolutionary pathway leading to cancer, and it is that head start which can be the basis of a dominantly inherited cancer susceptibility.

Knudson made the additional suggestion that a further genetic step was needed somatically, which knocked out the activity of the remaining normal

gene. Thus, while the familial inherited susceptibility was dominant, the genetic event that contributed to tumour progression at the somatic level was recessive. These ideas, therefore, predicted a class of recessive genetic changes in tumourigenesis whose normal functions were a block to the development of a cancer. As a result, these genetic changes, which are in contrast to the dominant oncogene mutations identified originally through studies of the oncogenic retroviruses, have sometimes been called tumour suppressors.

Familial adenomatous polyposis: a model tumour suppressor

Familial adenomatous polyposis (FAP), which I have studied with colleagues at St Mark's Hospital (London) and elsewhere, and in my laboratory, for a number of years, provides a classic example of an inherited susceptibility to a tumour due to mutation in a tumour suppressor gene. I hope that William Harvey would have appreciated the importance of studying this comparatively rare disease. Thus, in a letter to a Dutch physician, William Harvey said 'nor is there any better way to advance the proper practice of medicine and to give our minds to the discovery of the unusual law of nature, than by careful investigation of cases of rarer forms of disease' [8].

Though familial adenomatous polyposis, or APC as it is often called, was first described late in the 19th century, it was at St Mark's under the guidance of Lockhart-Mummery in the 1920s that it was first clearly defined as a Mendelian dominantly inherited disease. Affected individuals usually develop from a few hundred to several thousand adenomatous polyps in the colon and rectum, starting usually in their early teens. If left untreated, one or more colorectal carcinomas will inevitably arise in the third or fourth decade. Total colectomy removes the major part of this risk if carried out sufficiently early, but leaves the risk of certain other manifestations, including upper gastrointestinal tract tumours and desmoids, which are a serious cause of mortality.

The clue to finding the position of the APC gene came from a single case report by Herrera and colleagues in Buffalo, United States, who identified an individual with polyposis who apparently died from its complications, but who in addition had a number of other developmental abnormalities [9]. This suggested the possibility of a chromosomal abnormality and, as a result, led to the identification in this patient of a deletion of one band of chromosome 5, namely 5q21. From this clue, and using the families accumulated over many years at St Mark's and elsewhere, we were able, using by now standard DNA techniques, to confirm that the APC gene indeed resided at position 5q21 [10]. The APC gene was then identified, using positional cloning techniques, by Nakamura and others through an even more fortunate inherited deletion which, because it was much smaller, pinpointed more accurately the position of the gene [11,12].

APC is a relatively large gene, coding for a 2843 amino acid protein. To prove its role in polyposis it was necessary to identify mutations that explained the inheritance of the disease in families. It is still not a trivial task to scan a DNA stretch of nearly 9,000 base-pairs for the single change that may knock out the gene function and explain the inheritance of polyposis. The earliest results already indicated that the vast majority of mutations did knock out the function of the APC gene, and also revealed similar mutations in sporadic colorectal cancers which had nothing to do with the inherited form of the disease, thus directly confirming Knudson's far-sighted ideas. Ironically, one of the most powerful techniques for identifying mutations which knock out the function of a gene, is to study *in vitro* the protein that should be made by a segment of the gene delineated by the polymerase chain reaction. Truncating mutations within such a segment lead to a smaller than expected protein product whose size indicates directly the position of the mutation.

Several hundred APC mutations have now been sequenced, both in individuals with APC and in sporadic colorectal carcinomas. These data confirm APC as a tumour suppressor since more than 95% of the mutations identified are truncating and so knock out the function of the protein, and up to 70% or 80% of sporadic colorectal cancers have mutations similar to those found in individuals with the inherited disease [13]. The data also show, somewhat surprisingly at first, that different mutations may give rise to clinically distinguishable forms of polyposis. Thus, some mutations are associated with a much larger number of polyps and more of the additional manifestations of polyposis (such as upper GI tumours and desmoids) than other mutations which give rise only to hundreds rather than thousands of polyps, and seem to have a slightly less severe phenotype [14]. In addition, it has become clear that there are significant portions of the gene where mutations at most give rise to very mild forms of the disease, sometimes called attenuated adenomatous polyposis coli. These data are explained by the fact that the protein product functions as a dimer and that hetero-polymers formed of mutant and normal forms of the protein may function at a near normal level, provided mutations occur sufficiently far along the gene towards its 3' end, or can be bypassed by alternative mRNA splicing of exons at the 5' end.

The pattern of sequence changes found in the somatic mutations in sporadic colorectal cancers, which includes a mixture of predominantly small deletions and a variety of single base pair changes, does not indicate any major role for external mutagens in the genesis of colorectal cancers [13]. This is particularly significant since several lines of evidence indicate that the APC mutations, when they occur, may be the earliest events leading to the initiation of a tumour. The p53 gene, which is the mutated gene

most commonly found so far in all human tumours, is mutated in approximately 50% of colorectal carcinomas. The DNA sequences of these mutations support the view that comes from the analysis of the APC mutations, namely that external mutagens are not important even for the relatively early stages of the development of the tumour. In contrast, the p53 mutations found in lung cancer are just those that would be expected from the mutagenic effects of the hydrocarbons in cigarette smoke, providing some of the most direct evidence for the role of smoking in the cause of lung cancer [15].

An analysis of the germline mutation DNA sequences found in the APC gene provides, for the first time, a basis for the proper estimation of DNA-based germline mutation rates in humans. The data suggest that the mutation rate at the germline level for simple single-base pair changes is of the order of 5×10^{-9} per generation, while particular short repeated sequences may have mutation rates that are up to a thousand times more frequent than this [10].

The protein sequence of the APC gene product, derived from its DNA sequence, did not give any immediate clues as to its function. More recent studies, however, have shown that the APC protein is involved in a complex of molecules that appears to control cell-cell and cell-basement membrane attachment, and the signalling processes contingent on this attachment which control cell differentiation and division. Intriguingly, an analogous signalling pathway has been described in the geneticist's classical model, the fruit fly Drosophila, showing once again the value of basic studies in model organisms using the powerful modern DNA-based technology [16]. The overall data clearly indicate that interference with cellular attachment is a key early stage in the development of an epithelial tumour, where independence of growth from the constraining effects of attachment between cells and to the basement membrane appears to be the first hurdle that must be overcome for the initiation of a tumour.

The discovery, in collaboration with Shirley Hodgson and Nick Wright, of a unique individual who was an XO/XY chromosomal mosaic as well as having polyposis, enabled Marco Novelli in the Cancer Genetics Laboratory at ICRF to show clearly that very early polyps in the polyposis patient are mostly polyclonal [17]. This striking result, which was predicted by earlier genetic studies of FAP polyps, indicates that a single abnormal or malfunctioning version of the APC gene may be enough, perhaps through a gene dosage effect, to initiate a small adenomatous growth. Soon after, however, there is strong selection for loss of the remaining functioning APC gene, as indicated by the fact that a very high proportion of sporadic colorectal carcinomas carry only mutant, non-functioning versions of the APC gene. It is then, within this favourable growth environment, that the opportunity arises for the occurrence of further mutations that are selected to form the basis of the progression from adenoma to carcinoma.

There is now a mouse model for the human APC mutations. Thus, the multiple intestinal neoplasia (MIN) mouse, identified by Amy Moser and Bill Dove in the United States as a product of chemical mutagenesis, is a nonsense mutation in the mouse version of the APC gene with closely similar consequences to the mutations found in polyposis patients [18]. There are, however, significant differences: the MIN mouse has more polyps in the small than in the large intestine; it was identified because of the anaemia that results from loss of blood through the tumours; and it generally dies of intestinal obstruction before the development of frank carcinomas. Nevertheless, the MIN mouse provides a valuable model for the experimental study of the control of colorectal tumourigenesis. Using it, we have been able, for example, to show the direct effect of increasing fat in the diet on increasing the number of tumours (Wasan, personal communication). This effect seems to depend on the microbial flora in the intestine. The result provides strong support for an interaction between fat in the diet and the gut flora as a contribution to the development of human colorectal carcinomas. This provides a much more direct explanation for the evidence of the role of diet in colorectal tumours than comes from even the most careful case-control or prospective studies. Combining the MIN mutation with mouse versions of other mutations known to occur in human colorectal cancers should make it possible to provide an appropriate model of *spontaneous* tumourigenesis in the mouse. This will surely then provide the best basis for the study of environmental factors in cancer as well as trying out and testing novel therapies.

Cell division and death: models for the cancer process

In addition to the p53 mutations found in colorectal carcinomas, the key signalling oncogene, *ras*, is also frequently mutated. More recently, the study of other colorectal cancer families, including in particular those in which colorectal cancer occurs together with several other cancers, has uncovered a different class of gene defects involved in the repair of mismatches between the DNA helices which often result from errors occurring during DNA replication.

Colorectal cancer provides an excellent model for study, particularly because of the knowledge about familial forms and also because the pathway from adenoma to carcinoma is so clearly defined and often available for study. Similar pictures concerning genetic changes in many other cancers are now appearing.

There have been many attempts at formulating quantitative models for the initiation and progression of cancers. Perhaps the best known is the use of the age-incidence curve for the onset of cancer to estimate, somewhat crudely, the number of steps that may be involved in the complete process. Thus, based on a

model of constant exponential growth with one mutation replacing another, so that the time taken before a cancer is initiated is determined by the number of events needed, Armitage and Doll in 1954 estimated that there were between six and eight steps [19]. The difficulty with these models is that they consider only cell division and not cell death, they do not adequately take selection into account and, because they are based on exponential growth, they do not easily explain either the long lag period (often up to 20 years) before many epithelial tumours such as colorectal carcinomas develop, nor do they account for benign growths.

Any population growth is a balance between the birth rate and the death rate, and this applies as well to the growth of a population of cells forming a tumour as it does to the growth of human populations. The death rate may often be as important in controlling population growth, if not more so, than the birth rate. Until comparatively recently most of the focus on the role of genetic changes in cancers, especially oncogenes controlling growth and differentiation, has been on the stimulation of the growth rate, namely the birth process. Only recently, particularly through work on the function of the p53 gene, has it been realised how important genetic changes are in cancers that influence the death rate. Thus, the particular role of the p53 protein appears to be to recognise errors in the DNA, most probably breaks, and to signal the arrest of cell division until the DNA errors are repaired [20]. This is presumably in order to prevent the accumulation of DNA mutations which are clearly detrimental. If the number of errors in the DNA is overwhelming, and therefore the level of bound p53 is too high, a signal is given to the cell that it should commit suicide. This is the process of 'apoptosis' or programmed cell death, first clearly defined by Alastair Currie and Andrew Wyllie in 1972. This default pathway for controlling repair of DNA lesions ensures that, if their number is too large for them to be effectively repaired, then the cell is doomed to commit suicide. The work of Tyler-Jacks using mice for whom both p53 genes have been knocked out, has clearly indicated that the role of p53 mutations may be to interfere with and so hinder the normal process of programmed cell death [21]. This suggests, therefore, that p53 mutations are selected for because they reduce the probability of cell death, not because they increase the rate of cell division. The effects of mutation in the mismatch repair genes may well lie along a similar pathway.

It is becoming clear that genetic changes influencing the probability of apoptosis are at least as important in the progression of cancers as those that control persistence of growth and the rate of cell division. Furthermore, these ideas suggest that most current cancer therapy, either x-rays or chemotherapy, induces apoptosis and cell death by suicide rather than by direct killing. Therefore, basing therapeutic regimes on these ideas might well improve their efficacy.

In collaboration with my colleague Ian Tomlinson, I have been formulating a model for cancer progression which takes into account cell birth and death rates as well as the probability of differentiation, using colorectal cancer as our template [22].

Colorectal crypts, which are clonal, [17] have at their base a stem cell population which divides and differentiates to give rise to the other cells of the crypt. The stem cells produce an intermediate proliferating zone of cells half way up the crypt. This differentiates terminally before it reaches the luminal surface where it is destined to die, probably by apoptosis, and be shed into the lumen. Stem cells and the cells in the intermediate proliferating zone may renew by reverting to another division, differentiate to the next stage, or die by apoptosis (see Fig 1). In the normal bowel the stem cells must exactly renew themselves, otherwise crypts, and so the bowel itself, would expand without limit. In contrast, the intermediate proliferating cells do not sustain their number by division, otherwise they would themselves be stem cells. At the normal equilibrium, the ratio of stem cells to intermediate proliferating cells to fully differentiated cells may be of the order of ten to a hundred to a thousand respectively. These relative proportions will, for the most part, remain stable in spite of the enormous rate of turnover—every few days—of the surface of the colon and rectum. Mutations affecting either the death or the differentiation rate of the intermediate proliferating population of cells may often result in a new equilibrium, provided the effects of the mutation are not too large. Thus a series of mutational steps can take place, each time increasing the number of intermediate cells relative to stem cells but not yet leading to exponential growth (Fig 2). The more such steps have occurred, the higher the probability that the next change will lead to exponential growth and so to the development of a cancer.

This model has major implications for understanding the nature of tumour progression. First of all, it explains the occurrence of a benign phase associated with a finite increase in size before exponential growth is initiated. Second, it can account for the long lag phases before the final development of a cancer without the awkwardness of having exponential growth at very low rates. Thus, at each new plateau a considerable period of time may elapse before the next mutation arises which provides the selective basis for a further increase in the size of the tumour. It must be emphasised that at no time is the population of cells static. It is continually turning over, providing extensive opportunities over many, many cell generations for the production of appropriate mutations with the selective advantage necessary to take the tumour to its next stage. Sometimes, perhaps either by chance or because of the nature of the tissue, or because of the sequence of mutations that has been selected for,

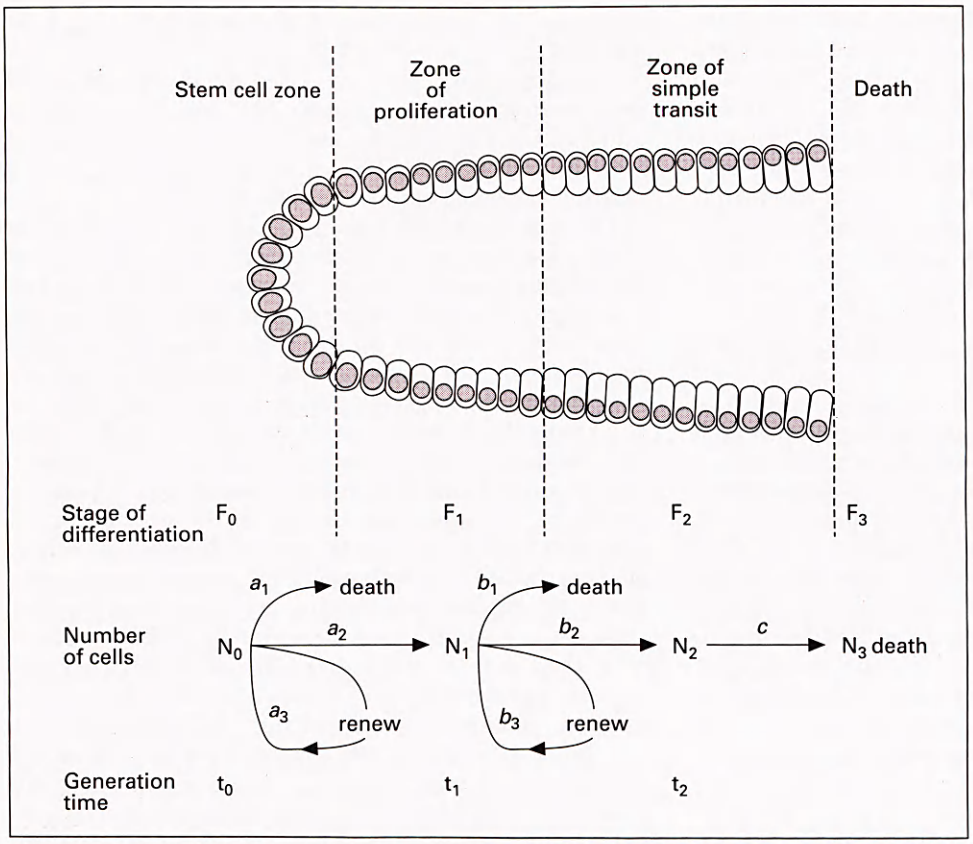


Fig 1. Model of cell division, differentiation and death in the colonic crypt. a_1, a_2 and a_3 are the rates of stem cell death, differentiation to proliferating zone and renewal, respectively. b_1, b_2 and b_3 represent rates of death, differentiation to transit and renewal for the proliferating zone cells. Normally, $a_1 = 1/2$, for precise stem cell renewal, and $b_3 < 1/2$, to limit turnover of proliferating zone

there may not be the opportunity for a further change leading to exponential growth. In that case the end result is a benign tumour that never becomes malignant.

Could this model of somatic mutation be relevant to diseases other than cancer? Some years ago, for example, Earl Benditt described the clonality of atherosclerotic plaques, an idea that seemed totally heretical but has, I believe, subsequently been confirmed. Perhaps such a plaque is analogous to a small benign lesion that never acquires the propensity to progress. Could this sort of change also be applicable to some cases of sporadic Alzheimer's disease or the non-inherited forms of Creuztfeldt-Jakob disease? Another area which may be analogous with Knudson's model for cancer is the genesis of congenital malformations. Is it possible that early genetic changes during embryological development could give rise to congenital malformations which may have their counterpart in a rare inherited syndrome but, as in the case of the atherosclerotic plaque, never progress to malignancy? Our model could fit this situation precisely, and suggests a search for genetic changes in the key tissues involved in a particular congenital malformation, using the same approaches that are used for studying cancers.

A framework for understanding cancer

These ideas on the somatic evolution of cancer and the molecular and cellular data which support them, now provide a basic framework for understanding the initiation and progression, at least of epithelial tumours. The earliest mutation, perhaps associated with the APC gene, facilitates independence of growth by loosening or abrogating the attachment of cells to each other and to the basement membrane. Further changes in adhesive properties of the cells, for example, affecting E-cadherin, the key molecule for epithelial cell-cell attachment, will enhance this process. Subsequent changes increase the growth rate by affecting growth factors, their receptors and the signalling processes to the nucleus that control both cell division and cell differentiation. One or more of these changes is likely to be associated with an increase in the frequency of programmed cell death as, for example, is

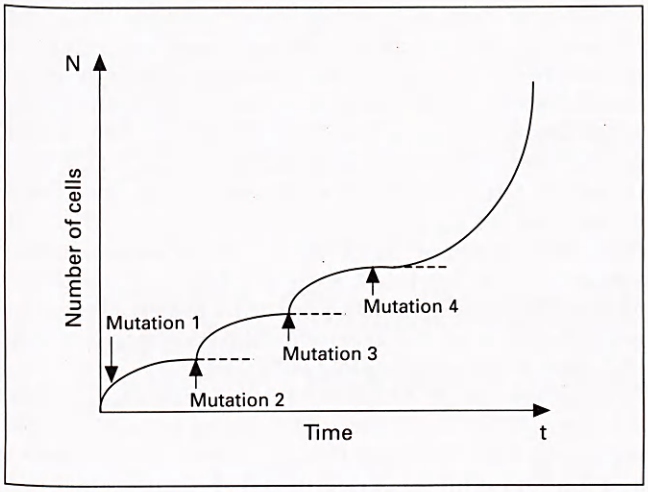
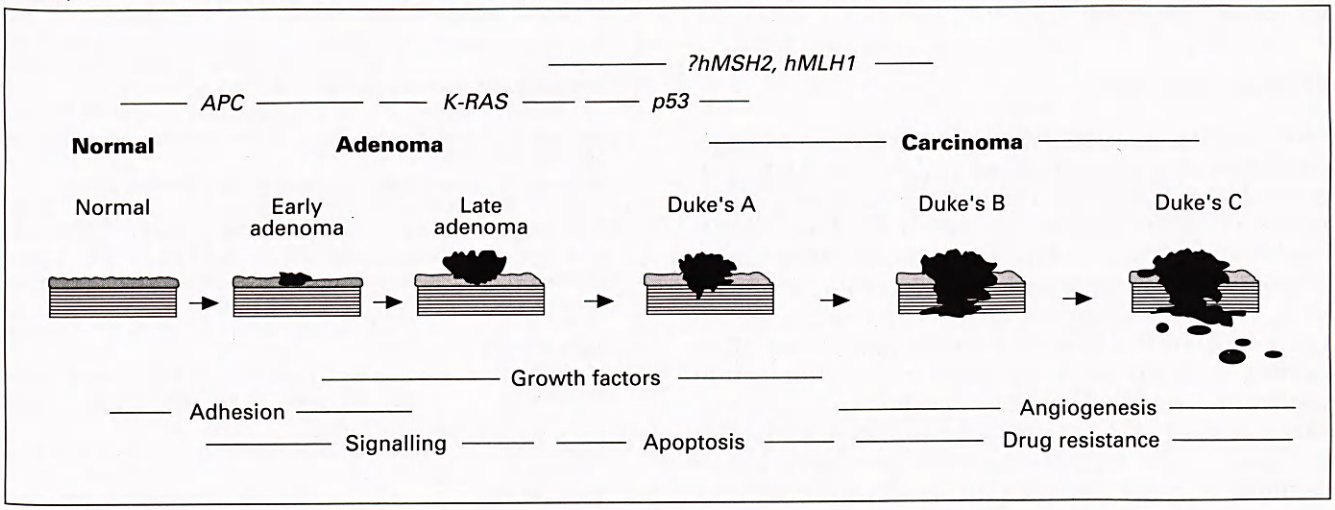


Fig 2. Successive mutation and selection steps for increasing levels of the number of intermediate proliferating zone cells to successive equilibria before onset of exponential growth

known to be the case when epithelial cells become detached from the basement membrane. This then places a premium on selection for mutations that inhibit or block the suicide pathway, such as p53, and perhaps mutations in the mismatch repair genes. Other mutations may affect the genes that control these steps in cell division more directly. Later changes in cell surface and growth properties then promote metastasis, and the angiogenesis that is needed to feed a secondary growth. Still later genetic changes lead to resistance to naturally developed anti-cancer immunity, as well as resistance to currently available therapy, especially using cytotoxic drugs. The early disruption of tissues associated with changes in adhesive properties of epithelial cells may be enough to give an analogue of an inflammatory response that promotes some vascularisation, which in turn may be enough to sustain the early stages of tumour growth. That is why I believe that strong selection for angiogenesis is probably a somewhat later event (see Fig 3).

Cancer is largely environmentally determined, as is most clearly illustrated by the now classical surveys of Richard Doll and Richard Peto [23]. The framework for understanding cancer, which enables the construction of appropriate animal models, provides a basis for direct experimentation on environmental effects—the diet in particular. But there is still room for some multifactorial inherited susceptibility that is more prevalent than the rare, but highly informative, mutations which give rise to polyposis and other clearly inherited cancer syndromes. A discourse on the approaches to using genome analysis to identify specific genes involved in multifactorial susceptibility could itself be the subject of another Harveian Oration. Suffice it to say that the technology is there to identify low penetrance, relatively high frequency genes which could have an important influence on the chances of getting different types of cancers. Apart from conventional linkage studies and general analysis of somatic genetic changes in tumours, a key to the identification of such genes will be intelligent guesses at candidates. These can then be tested for their effect on cancer incidence through straightforward case-control studies. Many of the clues for such candidates may come from finding the genetic steps that determine the somatic evolution of a cancer. Others may relate to systemic effects, such as the HLA genes which control immune response, or genes controlling skin colour and complexion, which must be the major factors determining the incidence of skin cancers, including melanoma.

Fig 3. A scheme for the adenoma to carcinoma progression, indicating, above, the stages at which mutation in the *APC, K-ras, p53, hMSH2* and *hMLH1* genes may occur, and below, the key functions being selected for at different stages



Applications

The ultimate aim of even the most basic cancer research must surely be to increase opportunities for prevention, early detection and effective treatment and cure of cancer. The discovery of genes for inherited cancer susceptibilities enables effective genetic counselling to be provided through the establishment of cancer family clinics. Monoclonal antibody reagents produced in laboratories are now becoming part of the armoury for routine pathological diagnosis. Approaches are already being worked out for the early detection of cancer, using as its key signature the genetic changes which I have described. For many cancers, screening a blood sample using sensitive DNA techniques to identify key oncogene mutations may become an effective method of early detection. While such procedures are sophisticated as far as their development is concerned, their application through the use of DNA in diagnostics is no more expensive, and probably less so, than many of the routine measurements that are now part of standard clinical practice. Even ABO and Rh blood typing are now done using DNA tests rather than classical serology.

Current therapy should be improved, based on the ideas of apoptosis, and novel, more specific, imaging techniques, especially using monoclonal antibodies, are beginning to find their way into standard practice. Molecular approaches have revolutionised our understanding of the immune response and opened up the possibility of effective immunotherapy directed against the changes in cancers which we can now identify at the DNA level. Each genetic step provides a new opportunity for the development of assays to identify more specific drugs for the treatment of cancer. New, improved animal models, based on genetic understanding, provide a more effective basis for exploring dietary and environmental factors in the genesis of cancer, as well as for testing new forms of therapy. The list of possibilities is enormous, and it would take an extraordinary pessimist not to expect major advances in applications from the laboratory to the clinic within the foreseeable future.

Costs and priorities

The question inevitably arises as to the cost of novel procedures for prevention, early detection and treatment. Here, I tread on the most dangerous ground, but one which I believe has not yet been adequately covered by anyone so far. Detailed cost effectiveness models, for example for colorectal screening, come up with relatively modest costs per cancer death prevented on the basis of current approaches [24]. Adding even the most sophisticated early detection procedure would, I believe, be a marginal extra to these costs. I also wonder whether such estimates adequately take into account the very high cost of terminal treatment of a cancer, let alone the value of prolonging and preserving the quality of life. The pressure for application of a successful procedure is bound to lead to reduction in costs through automation and greater efficiency of implementation, while the costs of the care of the infirm elderly will undoubtedly continue to soar. Of course, each new proposal must be carefully evaluated in this era of cost conscious and evidence-based medicine. But the real decisions will not lie there. They will involve some consensus on just how much society is willing to pay overall for health care costs, and how we can learn to enable people to live a comfortable and high quality life until they die at a suitably old age.

Whatever else is done, we must at least ensure that the opportunity to do first-class clinical research in the setting of our National Health Service is preserved and that the training of doctors for the future does not discourage participation and training in research, just at a time when it is most productive. Only in this way can we assure effective translation of advances from the laboratory into the clinic.

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