GENEWATCH
James A. Morris, Consultant Pathologist
Lancaster & Kendal Hospitals

SUMMARY

The process of ageing and the development of cancer are closely related and depend on the accumulation of DNA damage and gene mutation in somatic (body) cells. Somatic mutation can arise spontaneously, as a result of normal cellular metabolism, or can be caused by radiation, chemicals or viruses. In view of this a preventive strategy is proposed based on developing methods to measure the rate of somatic mutation and then applying the methods to monitor selected groups in the general population. An increased mutation rate would be investigated with a view to recognising the environmental cause and removing or reducing it. The long term aim of the programme would be to reduce the somatic mutation rate in the general population and thereby reduce the incidence of premature death from cancer and other degenerative diseases and produce a longer and healthier lifespan.

INTRODUCTION

In recent years the mechanisms leading to cancer have become much more clearly defined. Each cell in the body has approximately one hundred thousand genes and up to a hundred of these are concerned with growth control. One set of genes, called proto-oncogenes, code for proteins which stimulate cell division. Another set of genes, called anti-oncogenes or tumour suppressor genes, code for proteins which suppress cell division. These sets of genes normally act in a coordinated way to control cell growth but direct damage to the genes can lead to inappropriate expression with uncontrolled cell growth and the development of cancer.

In most cases damage to a gene renders it inactive, but rarely only control sequences are damaged and when this occurs the gene might be inappropriately expressed. In theory cancer could arise due to direct damage to a single proto-oncogene or due to inactivation of two anti-oncogenes. In the former case the damaged gene would stimulate cell division, in the latter case both the maternally- and paternally-derived anti-oncogenes would need to be damaged to remove growth suppression. In practice, however, it is found that in most cancers more than one proto-oncogene system needs to be activated or more than one pair of anti-oncogenes needs to be inactivated. It is easy to demonstrate that this must be the case. There is a one in ten million chance of a single anti-oncogene undergoing mutation with each cell division, but there are seven million billion cell divisions in a human lifetime. Thus if only one or two mutations were required for cancer we would all die of this disease in childhood. If four mutations are required for cancer, however, most of us will escape the disease in a seventy-year lifespan unless we are subjected to some environmental insult which increases the somatic mutation rate.

Somatic mutation can arise in a number of ways:

1 Spontaneous mutation
During normal cell metabolism reactive chemical compounds are produced which can damage DNA. The majority of the injuries are repaired by special enzymes but the repair process is fallible and occasionally permanent damage to DNA results. The spontaneous mutation rate is of the order of one in ten million per gene per cell division and one in ten million per gene per annum in non-dividing cells.

2 Radiation
High dose ionising radiation can damage DNA leading to mutation and an increased risk of cancer. This has been shown in patients treated with radiation for ankylosing spondylitis and in the survivors of nuclear bomb explosions in Japan. There is currently much interest in the role of low dose ionising radiation in mutation and human cancer particularly in relation to residence close to nuclear installations and to radon levels in the home. It is clear that even natural background radiation will contribute to somatic mutation but the degree of that contribution has not been established.

3 Chemicals
Many chemicals can react with and damage DNA leading to somatic mutation. Some of these chemicals are widespread in the environment, albeit at low doses, and could make a significant contribution to human cancer. One environmental chemical carcinogen, ie tobacco smoke, certainly does.

4 Viruses
Viruses have their own genes made of DNA or RNA. When they infect cells it is possible for the viral genes to be inserted into the host genome. This can damage the host genome at the site of insertion and interfere with control sequences for genes at more distant sites.

The basic concept, therefore, is that somatic cells accumulate DNA damage throughout life due to spontaneous mutation and the effects of radiation, environmental chemicals and repeated viral infections. If one of our cells by chance acquires several key mutations in growth control genes then it will divide out of control and produce cancer. Furthermore the chance of cancer arising will increase with age because the probability of a cell having acquired several mutations is much higher in old age than in youth. Thus cancer can be regarded as part of the process of ageing and there is now a strong body of opinion that ageing itself is a consequence of accumulated DNA damage leading to progressive cell dysfunction and a rapidly rising chance of other diseases such as heart disease, degenerative lung disease and infection.

If these concepts are correct then a logical strategy would be to develop methods to measure the rate of somatic
mutation and then use them to monitor the population in order to detect and hopefully prevent environmental factors which increase the mutation rate. This is the basic strategy of Genewatch.

METHODS

There are a number of ways in which DNA damage can be measured in somatic cells.

1 Chromosome structure

One approach is to produce chromosome preparations and to count the number of structural abnormalities. The preparations are made by growing lymphocytes in culture, arresting the cells in metaphase, disrupting the cells by osmotic swelling and then fixing and staining the chromosomes on glass slides. The chromosome spreads from several hundred cells are then examined by light microscopy and the number of structural abnormalities recorded. The process, however, is time-consuming, it requires a highly skilled observer, the assessment is subjective and only gross chromosome abnormalities can be detected. It is possible, however, that the sensitivity could be improved by using more advanced optical techniques such as scanning electron microscopy or confocal laser microscopy.

A variation on this approach is to measure sister chromatid exchange in chromosomes. A metaphase chromosome is composed of two identical (sister) chromatids held together by a centromere. Some exchange of DNA sequences between the two chromatids occurs normally and can be visualised by special staining techniques. The rate of exchange is increased with increased DNA damage.

2 Lymphocyte clonal assay

The locus of the gene that specifies the enzyme hypoxanthine phosphoribosyl transferase (HPRT) is situated on the X chromosome. Each somatic cell has only one functional X chromosome as the second X chromosome in female cells is inactivated early in embryogenesis. It is possible to detect lymphocytes which have undergone mutation at the single HPRT locus because in the absence of the enzyme the cells will grow in a culture medium containing 6-thioguanine, a substance which poisons normal lymphocytes. The lymphocyte clonal assay therefore involves placing a large number of lymphocytes onto a culture medium with 6-thioguanine and counting the number of colonies which are produced. Each colony is the progeny of a single mutant cell.

It has been found that normal adults have approximately five mutant lymphocytes per million cells. There is a detectable increase in people who smoke and the mutant frequency rises by approximately 1% per annum in adult life. Furthermore it has recently been shown that there is a detectable increase in mutant frequency in people exposed to increased indoor radon levels. This method obviously has significant potential for monitoring selected groups in the population but it is expensive and 80ml of blood is required for the test which in practice means that it is limited to use in adults.

3 Histochemical staining of the human colon

Human colonic mucosa shows an interesting staining pattern which can be used to measure somatic mutation. Individuals who lack the enzyme o-acetyl transferase (oat) show positive staining of their colonic epithelial cells with a certain histochemical technique, but those who possess the enzyme have a negative result. Those who lack the enzyme are homozygous for the defect having inherited defective genes from their mother and father (oat/-oat-). Those who possess the enzyme are either homozygous for the gene (oat+oat+) or heterozygous (oat-oat-). The latter have inherited a functioning gene from one parent and a defective gene from the other. In the United Kingdom 40% of the population are heterozygous.

There is evidence that all the cells in a singlecrypt of the colon are produced by a single stem cell. If that stem cell in a heterozygote undergoes mutation from (oat+oat-) to (oat-/oat-) then there will be isolated staining of the crypt epithelium. Thus by staining the colonic mucosa of heterozygotes and counting the number of positive staining crypts it is possible to measure the mutant frequency in colonic cells. The recorded frequency in studies so far is in the region of one mutant per ten thousand. This is a higher rate than found in lymphocytes and possible explanations include

1 there is a higher rate of mutation in colonic cells due to the composition of gut contents
2 there is higher cell turnover in the colon with more cell divisions
3 specimens of colon are only available from patients who have disease
4 the change from the heterozygote to the homozygote may be due to loss of part of a chromosome with reduplication of the remaining chromosome which occurs more commonly than single gene mutation

This technique is of no value for population monitoring but it does show that it is possible to measure somatic mutation in individual tissues.

4 Red cell antigens

An approach with great potential is to measure the frequency of loss of antigens from the red cell surface. For instance people with the blood group MN have M antigens and N antigens on their red cells. The gene specifying the M antigen is inherited from one parent and the gene specifying the N antigen is inherited from the other parent. Somatic mutation in red cell precursors, however, will lead to loss of the M antigen or the N antigen from some of the cells. There are a number of ways in which this tiny subset can be measured. One approach is to absorb out the red cells bearing M antigen using an anti-M antibody and then count the nonabsorbed cells. Another approach is to label the M-bearing red cells with an anti-M antibody and then attempt to separate labelled and unlabelled cells using a flow cytomter. These techniques are at an early stage of development but they hold great promise for population monitoring as a single ml of blood contains five billion red cells.

PLAN OF INVESTIGATION

The Genewatch programme will be run by scientists at Lancaster University working in association with clinicians from Lancashire and Cumbria. Part of the programme will be concerned with developing methods to measure somatic mutation, building on the work that has already been done. Lancaster University is fortunate in having an excellent electron microscopy unit in the Department of Biological Sciences which is well placed to improve methods of structural analysis of chromosomes. In addition the development of methods using red cell antigens will have a high priority and this will be carried out in association with
scientists at the Lancaster Blood Transfusion Centre who already have considerable expertise in this field.

In parallel the lymphatic clonal assay for mutation at the HPRT locus will be used to measure the mutation rate in selected populations. The following are some examples of studies that will be undertaken:

1 The lymphocyte mutant frequency will be measured in a group of smokers and age-matched controls to validate the method in our hands.

2 A group of patients presenting with breast cancer will be compared with age-matched controls. If the breast cancer patients have an increased mutant frequency then their lifestyles will be analysed to find the possible causes. Any hypothesis of cause will be tested using independent groups and controls matched for other variables.

3 Work will be done to see if radon levels in homes in this area have a measurable effect on the mutant frequency.

4 Work will be done to see if living in the vicinity of nuclear establishments has a measurable effect on the lymphocyte mutant frequency.

5 The lymphocyte mutant frequency will be measured in patients presenting prematurely with other diseases of ageing such as ischaemic heart disease.

6 The role of increased somatic mutation in male infertility will be investigated.

When the methods have been improved so that less blood is needed then a study of the mutation rate in cord blood will be undertaken in order to study effects in utero.

DISCUSSION

The programme of research outlined in this article is very ambitious. The aim is to reduce the rate of DNA damage and thereby slow the rate of ageing, reduce the incidence of premature death and produce a longer and healthier lifespan. Recent advances in molecular biology have given a much clearer understanding of the processes which lead to cancer and which cause ageing and have shown that these goals are attainable. It is important to stress, however, that the programme is not just concerned with diseases of old age. The central concept is that DNA damage occurs throughout life and the impairment in cell function that it produces will increase the risk of disease at all ages. Indeed the process of somatic mutation starts in utero and congenital abnormalities and childhood cancer are some of its consequences.

The fact that methods already exist to measure the somatic mutation rate also indicates that the aims of the programme are realistic. The lymphocyte clonal assay is sufficiently sensitive to detect the results of spontaneous mutation and therefore is of potential value for environmental monitoring. The assay, however, is expensive and time-consuming and requires 80ml of blood which limits its usefulness for investigating children. It is hoped that methods of assaying red cell antigens will be cheaper and more amenable for population monitoring.

This programme of research is particularly suitable for the new Lancaster University Medical Research Centre. It involves the development of methods at the university and their application to a broad range of diseases. This will allow very many clinicians in the area to be involved in this work. It is also particularly appropriate that a university in the north of England should pioneer this approach as the highest incidence of cancer and ischaemic heart disease is found in the north. Furthermore this approach to population monitoring will integrate with the work of the Environmental Epidemiology Unit in the Geography Department.

REFERENCES


Answer to quiz on page 222

An electron micrograph of a large cleaved lymphocyte (centrocye) from a case of diffuse non-Hodgkin’s lymphoma. The cleaved nucleus is vesicular and has a prominent nucleolus. The cytoplasm contains polyribosomes which are a marker of active protein synthesis.