Current topic

Oncogenes in malignancy

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All doctors are familiar with the meaning of the words oncology and genes but most are likely to have been quite puzzled over the last few years as they came upon the new hybrid term, oncogenes, and even more so by its siblings protooncogenes and cellular oncogenes. In this article I will attempt to explain what is meant by the term, how they have been discovered and defined, in what way they relate to normal human genes, how their study has shown the genetic basis of cancer, and make some suggestions for how their analysis could affect the diagnosis and treatment of cancers, particularly those of childhood. Unfortunately, most of this last section will be speculation and gazing into the future but in view of the tremendously fast rate of progress over the last five years it would be unwise to dismiss all such ideas as merely fantastical.

Identification of oncogenes

THE COMING OF CLONING

Oncogenes have been identified over the last few years by scientists working in the fields of virology, cytogenetics, and cell biology: all of them using the techniques of molecular biology and molecular genetics, which have been made possible by the development of recombinant DNA. The cloning of genes or pieces of DNA in recombinant molecules has made it possible to isolate and prepare in bulk DNA sequences that were previously present in such small amounts that they were impossible to purify. These purified sequences can then be used to probe for identical or related sequences in DNA from a variety of sources including tumours. The probing is made possible because the two strands of the double helix of DNA contain complementary sequences and when both probe and target DNA are melted to give single stranded molecules they will reform into a duplex wherever a complementary sequence is found (nucleic acid hybridisation).

The other technical advance which revolutionised the experimental approach to these problems was the discovery of a class of enzymes, found in

bacteria, which cut DNA at specific sequences of the base pairs (restriction enzymes). This gave the power to manipulate DNA very specifically and reproducibly.

Research in all the different and apparently non-related disciplines converged in a remarkable way to identify a few related genes.

THE ROLE OF THE VIROLOGIST

The study of tumour viruses in a range of animals (but not in humans until the recent discovery of the human immunodeficiency viruses) has thrown light on both the genes causing transformation and the mechanism by which this can arise.¹ Transforming retroviruses, which have a genome made of RNA rather than DNA, are found in a wide range of vertebrate species including monkey, cat, mouse, and chicken. The types of tumour they can cause also vary widely and include sarcomas, lymphomas, leukaemias, and carcinomas. Fortunately for the experimenter they transform cultured cells as well as rapidly inducing tumours in animals.

A virus normally contains only the genetic information needed for its own reproduction, which an infected cell will copy using its own machinery. An oncogenic virus also contains an 'oncogene', however, which has been transduced from the host organism into the viral genome at some previous point in its history. The structure of the gene will have been altered at the time at which it was picked up by the virus, any non-translated regions which interrupted the original gene on the chromosome (these are normally occurring and are called intervening sequences) will have been removed, and the gene may have been shortened or changed by point mutations. In some cases the virus may have picked up two such genes. It is the production of this abnormal protein by the host cell's machinery which converts it into a cancerous cell.

TRANSFERRING A TUMOUR GENE—THE CELL BIOLOGIST

When DNA extracted from human tumour cells is applied to a culture of the mouse cell line NIH 3T3

some of the cells take up small pieces of DNA and in around 15% of cases a small colony of cells grows out into a clump, a sort of mini tumour. The human DNA can be isolated from these cells by cloning and then analysed. In most cases the isolated gene belongs to a family (of three) genes called *ras* which had previously been identified as the oncogenes present in the mouse sarcoma viruses Kirsten and Harvey *ras*.² This provides very direct evidence that the alteration in a tumour cell is a somatic mutation of the cell's DNA.

THE CYTOGENETICISTS ART

As the methodologies for obtaining good chromosome preparations from tumour cells have improved a wealth of data have been produced on chromosome changes in tumours. In particular it has become possible to correlate chromosome changes with detailed diagnoses, progression of the disease, and secondary tumours. Amazing progress has been made in the cytogenetics of leukaemias and lymphomas but recently, with technical advances, many other tumours including those of soft tissue have begun to show consistent chromosome changes. The chromosomal changes observed may be translocations, gain or loss of a complete chromosome, or deletions. In the case of leukaemias and lymphomas, translocations have been particularly well defined and the hypothesis has been formed that it is the alteration in the structure or expression of the genes located at the translocation breakpoints which cause at least one step in the cell's progress to becoming cancerous. There has therefore been a belief that identification of genes located at these breakpoints will identify further oncogenes. This has

led to a surge of interest in human gene mapping with many valuable consequences. Because of the lack of resolution of most methods of gene mapping, however, it has been extremely difficult to implicate definitely many genes by these methods. The outstanding exceptions are the *c-myc* gene implicated in Burkitt's lymphoma and the *c-abl* gene implicated in chronic myelogenous leukaemia and acute lymphocytic leukaemia. Both of these will be discussed in detail later.

Cytogenetics has also played the major part in identifying the chromosomal position of and, more recently, facilitating the cloning of genes involved in cancers where there is an inherited predisposition. This will be discussed further in the section on retinoblastoma.

The nature of oncogenes

HAVE WE ALL GOT THEM?

The cloned oncogenes isolated by the methods described above can be used as hybridisation probes to search for the same sequences in DNA from non-tumour tissue. There were several surprises when this was done. It is clear that normal human DNA contains sequences complementary to the oncogene DNA and indeed these sequences are highly conserved throughout evolution. Both these findings suggest that they have normal cellular equivalents, performing essential functions of the cell (because of the high degree of species conservation). These normal equivalents of oncogenes are called cellular oncogenes or *c-onc* (undoubtedly a particularly misleading and confusing nomenclature) or protooncogenes. Thus the cellular equivalent

Fig 1 Relationship between protooncogenes, viral oncogenes, and activated oncogenes.

of the viral oncogene v-myc is called c-myc until the day when somebody identifies the real nature of this gene at which point it will revert to some quite straightforward biochemical name. Protooncogene conveys far more satisfactorily the sense that a gene has been identified, which when mutated in various ways has the potential to contribute to transformation of the cell. It is therefore the case that, to paraphrase Molière, we have all been inheriting oncogenes for years without knowing it.³ The relationships between the different forms are shown in fig 1.

WHAT DO THEY NORMALLY DO?

In only very few cases has the connection between a known oncogene and a biochemically defined human gene been made. This is achieved by comparing the sequence of a viral oncogene (for example) with newly acquired sequence data of human proteins and their complementary nucleotide sequences, making allowance for possible alterations and mutations of the sequence in the virus. In other cases the identity of the protein is still unknown but its site of action and other functional information may be known. The first oncogene whose cellular equivalent was identified was sis. In its viral form v-sis it is a simian sarcoma virus but its human equivalent has been identified as the β subunit of platelet derived growth factor. The table lists the other oncogenes with identified products. In general they appear to be growth factors and growth factor receptors, but any molecule exerting major control over cell growth or division can be considered a potential oncogene. Several excellent reviews expand this information.^{4 5}

HOW ARE THEY ACTIVATED?

At least two ways in which the protein amino acid sequence is mutated during oncogene activation are known. In the case of activated ras genes, isolated by transfection experiments, the mechanism is a simple point mutation leading to a single amino acid difference (fig 2). N-ras gene encodes a 21 000 dalton protein whose normal function is to couple

	ras protein p21			
	10 gly	11 ala	12 gly	13 gly
Normal	GGC	GCC	GGC	GGT
Bladder carcinoma			GTC val	

Fig 2 Mechanism of activation of the Ha-ras gene in bladder carcinoma.

growth factor receptors to inositol phosphate production.⁶ In the DNA isolated from tumours point mutations affecting amino acid residues 12, 13, and 61 have been found.

Secondly, a chromosome translocation such as the 9;22 translocation found in the Philadelphia chromosome in chronic myelogenous leukaemias can bring together parts of two genes that are normally quite separate in the genome. This can result in aberrant mRNA expression and splicing to include sequences from both sides of the breakpoint resulting in a novel chimeric c-abl protein with a molecular weight of 210 000 instead of the normal 145 000 and an altered amino terminal end.

In chromosome rearrangements such as the 8;14, 8;2, and 8;22 translocations found in Burkitt's lymphoma breakpoints occur around and adjacent to the c-myc oncogene altering the expression of the gene, probably by changing mRNA stability, rather than the sequence of the protein.

Over expression of an oncogene can occur because the DNA sequence is amplified many times. Amplification of several oncogenes has been shown but the most interesting, because of its clinical correlations, is N-myc in neuroblastomas. Again there is no change in the structure of the protein product.

Cancers and oncogenes

LYMPHOMAS, LEUKAEMIAS, AND TRANSLOCATIONS

Chromosome translocations are often found in leukaemias and lymphomas and by correlating all these findings at international workshops it is rapidly becoming possible to build up a consistent picture of specific translocations associated with particular tumour types.⁷ Some of the well established associations are shown in fig 3.

The value of these studies is twofold: from the clinical point of view it helps to define subgroups of disease with altered prognosis. Thus the child with acute lymphocytic leukaemia carrying a t(4;11) translocation has a particularly 'poor' prognosis. From the scientific point of view it defines the exact region of the chromosome where genes important

Table Identified oncogenes

Name	Human gene	Original virus
c-sis	Platelet derived growth factor	Simian sarcoma virus
c-erbA	Glucocorticoid receptor	Avian erythroblastosis virus
c-erbB	Epidermal growth factor receptor	Avian erythroblastosis virus
c-fms	Macrophage colony stimulating factor	Feline sarcoma virus

Fig 3 Specific translocations in malignancies of lymphoid lineage. CGL: chronic granulocytic leukaemia; CALLA: common acute lymphoblastic leukaemia antigen; Tdt: terminal deoxynucleotidyl transferase; ALL: acute lymphoblastic leukaemia; BL: Burkitt's lymphoma; and DR and B4 are cell surface antigens.

for at least one stage of the malignant process must reside. Several excellent reviews outlining the various chromosome changes are available.^{8,9} Recently considerable progress has been made in delineating the changes in solid tumours.¹⁰

Although it has been known for many years that the African form of Burkitt's lymphoma carries a t(8;14) translocation, more recently it was shown that variant forms of Burkitt's lymphoma existed with t(2;8) and t(8;22). In each case the breakpoint on chromosome 8 remained the same, at the position of the c-myc oncogene, and in each case the reciprocal translocation corresponds to one of the three immunoglobulin genes (heavy chain, κ light chain, and λ light chain). The DNA of the immunoglobulin genes rearranges to generate antibody diversity during the differentiation of B cells and detailed studies at the molecular level have shown that the translocation arises as a failure of the normal 'recombinase' mechanism. Not surprisingly the translocations in T cell leukaemias have been shown to involve the genes of the α chain of the T cell receptor which rearranges using the same mechanism during T cell development. In this case, however, the oncogene on the other side of the breakpoint has not yet been identified and a reversed genetic approach of cloning the DNA sequences across the breakpoint and then identifying their origin can be used.

Although the Philadelphia chromosome is a marker for chronic myelogenous leukaemia a cytogenetically indistinguishable chromosome is found in 2–10% of childhood acute lymphoblastic leukaemia (Ph⁺ALL). Molecular analysis has shown that as in chronic myelogenous leukaemia a

mutant c-abl gene is created by fusing sequences from chromosomes 9 and 22 but the position of the breakpoint on 22 is different,¹¹ although interrupting the same gene, and in this case the protein has a molecular weight of 190 000.

NEUROBLASTOMA AND GENE AMPLIFICATION

An amplified oncogene N-myc has been identified from neuroblastomas, both tumour tissue and cell lines. N-myc was identified both by weak hybridisation to the oncogene c-myc, because of some regions of sequence homology, and by molecular cloning of amplified sequences from a neuroblastoma cell line. The amplified sequences show up as homogeneously staining regions or double minute chromosomes in cytogenetic preparations from neuroblastoma lines. The frequency and degree of amplification in primary tumours varies greatly. Brodeur and colleagues showed that in 89 primary neuroblastomas amplification varied between three and 300 copies in 34 out of 89 tumours.¹² Amplification was not found in eight stage I or five stage IVS tumours, however, but was found in two out of 16 stage II, 13 out of 20 stage III, and 19 out of 40 stage IV tumours. Progression free survival also correlated with the amount of N-myc amplification. At 18 months, 70% of patients with one copy survived, 30% with three to 10 copies, and only 5% with more than 10 copies. Amplification of N-myc cannot be the primary cause of neuroblastomas as 62% of primary tumours have no extra copies. Its role is probably associated with the progression of the disease and this may be by a fairly general mechanism as amplification has also been found in retinoblastomas, small cell lung cancers, and malignant astrocytomas.

RETINOBLASTOMA AND DELETIONS

Retinoblastoma is representative of a class of embryonic tumours where loss of one of the pair of chromosome alleles by a variety of genetic mechanisms is essential to the development of the tumour.¹³ In about 5% of non-inherited forms of retinoblastoma a constitutional chromosome deletion of chromosome band 13q14 has been observed. In familial cases of retinoblastoma there is close genetic linkage between esterase D and the disease. Esterase D maps to 13q14. Both of these observations suggested that the gene predisposing towards retinoblastoma must lie in that band and (as originally proposed by Knudson¹⁴) loss of the wild type allele exposes the other mutant or deleted allele. This gene has now been cloned¹⁵ and shown to be a nuclear phosphoprotein associated with DNA binding activity.¹⁶ It is not clear that it can be classified as an oncogene, particularly as it appears to be loss of activity which causes the development of the tumour. However, it has increased our knowledge of the genetic mechanisms underlying cancer. Osteosarcomas are common as secondary tumours in cases of retinoblastoma and it has also been shown that chromosome 13 sequences are lost in these.

Wilms' tumour shows a similar relationship with sequences from band 11p13 but no candidate gene has yet been isolated.

Uses of oncogenes

PROBES FOR DIAGNOSIS

Once the breakpoint in a tumour has been defined and cloned hybridisation probes can be produced that are likely to detect rearrangements of the DNA in other tumours of the same type. This could be used diagnostically, probably rather more cheaply than by using chromosomes, or to look for residual disease. An example is the cloning of the sequence on the chromosome 22 side of the Philadelphia chromosome the so called bcr probe from the 'break-point cluster region'.

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TREATMENT??

Some examples will occur when study of the state, of either mutation or dosage, of an oncogene will indicate the prognosis in a particular patient and thereby influence the treatment regime. It may even be possible to design drugs which act against the altered specificity of a mutant oncogene or to regulate their expression by injection of an antisense RNA.

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