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CLINICAL ONCOLOGY

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C. J. WILLIAMS

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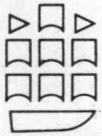

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CLINICAL ONCOLOGY

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Preface

This is the second edition in this series. The intervening years since the publication of the first have seen innovation and discovery in cancer diagnosis and management, marking cancer medicine as one of the fastest changing areas of modern medicine. This makes it also one of the more exciting. A book such as this cannot hope to cover such progress in any comprehensive sense. It is not intended as a textbook, but instead to whet interest in some of the facets of our subject where progress can be identified, and in these to give sufficient detail so that a sound understanding may be gained. Detailed referencing has been encouraged so that those who wish to scrutinise the original papers from which the content of these chapters are distilled may do so.

Time is a ruthless judge of scientific progress, allowing challenge of the most fundamental. Observing this challenge and the subsequent realignment of ideas contributes to our wisdom.

C.J.W.

Southampton, 1986 J.M.A.W.

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New developments

1. Oncogenes and the origin of cancer

Karen H. Vousden Christopher J. Marshall

INTRODUCTION

It has long been thought that neoplastic transformation involves genetic alterations. However, it has only been in the last eight years that we have begun to identify which genes are involved. The success of this undertaking has depended on the application of methods of isolating and studying specific genes (recombinant DNA techniques) to problems in tumour virology and tumour cell biology. In this review we shall discuss some of the contribution which such experiments have made to our knowledge of the origins of cancer.

VIRAL AND CELLULAR ONCOGENES

Although it is possible that many genes are affected in neoplastic transfection, it is most likely that many of these changes result from alterations to a small number of critical genes. We call these genes oncogenes. An oncogene is defined as a gene whose changed expression or altered product is *essential* to the production of the transformed phenotype.

Evidence that specific genes are involved in oncogenesis came originally from the study of viruses which were capable of inducing tumours in animals. Some of these viruses were found to have RNA as their genetic material and are known as retroviruses. The retrovirus genome is simple, consisting of three genes: *gag* and *env* which encode structural proteins, and *pol* which encodes the reverse transcriptase which makes a DNA copy of the RNA genome. However, examination of retroviruses which rapidly induce tumours in the animal host has revealed that these viruses have acquired additional sequences which are shown to be responsible for both induction of tumours and transformation of cells in culture. These sequences have been called viral oncogenes (v-*oncs*). In some viruses the v-*oncs* occur in addition to the normal viral sequences, whereas in others the oncogene replaces various portions of the viral genome. The importance of viral oncogenes to the study of human cancer became evident when it was shown that v-*oncs* were derived from cellular DNA sequences and that sequences homologous to these genes are also present in human DNA. The cellular genes are referred to as cellular oncogenes (c-*oncs*) or proto-oncogenes to indicate that they are not, as yet, transforming genes. Evidence that some of these cellular genes are transcribed in untransformed cells indicated that they have some normal function in the cell, but have the potential of inducing transformation after being incorporated in a retrovirus (reviewed in Bishop, 1983). Over 20 viral oncogenes have been identified so far (Table 1.1) and, with the possible exception of v-*rel*, human c-*onc* sequences homologous to each of the v-*oncs* have been described.

Table 1.1 Viral oncogenes

Oncogene	Species of origin	Protein product
abl	Mouse/cat	Tyrosine kinase
fps/fes	Chicken/cat	Tyrosine kinase
fgr	Cat	Tyrosine kinase
ros	Chicken	Tyrosine kinase
src	Chicken	Tyrosine kinase
yes	Chicken	Tyrosine kinase
fms	Cat	Tyrosine kinase structural domain
raf/mil	Mouse/chicken	Tyrosine kinase structural domain
mos	Mouse	Tyrosine kinase structural domain
erbB	Chicken	Tyrosine kinase structural domain (truncated EGF receptor)
sis	Monkey	Homology to PDGF
Ha-ras	Rat	Bind guanosine triphosphate
Ki-ras	Rat	Bind guanosine triphosphate
fos	Mouse	Nuclear location
myb	Chicken	Nuclear location
myc	Chicken	Nuclear location
erbA	Chicken	Cytoplasmic location
ets	Chicken	Cytoplasmic location
rel	Turkey	Cytoplasmic location
ski	Chicken	Cytoplasmic location

Although more v-*oncs* may yet be discovered, the number of oncogenes identified by viruses in this way would seem to be limited, since on a number of occasions the same oncogene has been transduced by different viruses.

Some of the proto-oncogenes have been shown to be very highly conserved across species barriers; for example the *ras* genes can be identified in human (Ellis et al, 1981) and yeast (DeFoe-Jones et al, 1983; Gallwitz et al, 1983). The strong conservation of proto-oncogenes in evolution is an argument that these genes have an important function in the normal physiology of cells.

Retroviruses can play a role in oncogenesis not only by transducing oncogenes but also by another route. Some retroviruses which do not carry an oncogene produce tumours only after a long latent period. For example, the avian leukosis virus (ALV) induces leukaemias in chickens by integrating close to and activating the proto-oncogene c-*myc* (Hayward et al, 1981). This elevation of transcription may result from the transcriptional control regions of the virus, the long terminal repeats (LTRs) providing a new promoter or enhancer element adjacent to the proto-oncogene. The enhancer activity of the viral LTR may be related to alterations in chromatin structure induced by ALV integration (Schubach & Groudine, 1984). ALV has also been shown to activate c-*erb*B by LTR insertion in avian erythroblastosis (Fung et al, 1983). Some mammalian chronic leukaemia viruses are also thought to act by proviral insertion and activation of cellular oncogenes. In cats feline leukaemia virus, (FeLV) can integrate close to c-*myc*, and in some naturally occurring cases may transduce the oncogene and contagiously transmit it between cats (Neil et al, 1984). In mice the integration of the murine mammary tumour virus (MMTV) may activate specific genes called *int*-1 (Nusse & Varmus, 1982) and *int*-2 (Peters et al, 1983). These two genes, *int*-1 and *int*-2, do not have homology to any of the known retroviral oncogenes demonstrating that the mammalian genome probably does harbour more potential

oncogenes than have been identified by the acutely transforming retroviruses. It is also possible that Epstein Barr virus, a DNA virus, may act as an insertion activator (Lasky & Troy, 1984).

Further evidence that cellular proto-oncogenes have the potential to transform cells came when cloned c-Ha-*ras* and c-*mos* were shown to transform cells once they had been put under the control of a viral LTR (Chang et al, 1982a; Oskarsson et al, 1980). Results such as these indicated that elevated levels of proto-oncogene expression are sufficient to transform cells. However, such structures seem to have a lower transforming efficiency than their viral counterparts and it is clear from sequencing data that all the viral oncogenes that have been studied in detail are structurally different from their cellular homologues (reviewed in Marshall & Rigby, 1984). It seems that qualitative, as well as quantitative changes, play an important role in the activation of a proto-oncogene.

EVIDENCE FOR THE ROLE OF ONCOGENES IN HUMAN MALIGNANCY

Although retroviruses carrying oncogenes have not yet been associated with human cancer, a number of lines of evidence have suggested an involvement of cellular oncogenes in human tumours. The role of these cellular genes has been demonstrated in three ways: by gene transfer (transfection) experiments, by examining chromosome aberrations in tumours, and by investigating expression of c-*oncs* in normal and tumour tissues.

Oncogenes detected by NIH-3T3 transfection assays

The transfection assay has identified genes in tumours which are capable of transforming recipient mouse fibroblast NIH-3T3 cells when the cells are treated with DNA from tumours (see Cooper, 1982; Land et al, 1983a). Using this technqiue, activated transforming genes have been isolated from a wide variety of human tumours and tumour cell lines (see Hall, 1984). Normal high molecular weight human DNA has never been shown to have transforming activity, even when isolated from normal tissue from a patient with a tumour which has an activated transforming gene (Santos et al, 1984). However, sheared DNA from normal chicken and mouse cells (Cooper et al, 1980) and from human lymphocytes (Schäfer et al, 1984) appears to be able to transform the NIH-3T3 cells at very low efficiency.

The transforming genes detected by the NIH-3T3 transfection assay fall into two broad classes. The first class are members of a single gene family, the *ras* genes, which are homologous to the oncogenes of Harvey and Kirsten murine sarcoma viruses. The second class of genes are not related to any of the known viral oncogenes and, unlike the *ras* genes, appear to be specific for certain tumour types (see Hall, 1984).

The human genome contains five known *ras* genes (Chang et al, 1982b; Hall et al, 1983; Shimizu et al, 1983). Two of these genes, c-Ha-*ras*-1 and c-Ha-*ras*-2, are closely related to the oncogene of Harvey MSV; two, c-Ki-*ras*-1 and c-Ki-*ras*-2, are closely related to the oncogene of Kirsten MSV; and the N-*ras* is more distantly related to the viral *ras* genes. Only three of these five genes, c-Ha-*ras*-1, c-Ki-*ras*-2 and N-*ras*, have been detected as activated transforming genes; c-Ha-*ras*-2 and c-Ki-*ras*-1 are pseudogenes and probably not expressed. The activated *ras* genes from a number of human tumours have been cloned and sequenced. All of the *ras* genes,

isolated from tumours so far, have been activated by a single point mutation altering the amino acid at either position 12 (glycine) or 61 (glutamine) in the 189 amino acid *ras* protein product (see Table 1.2). In vitro mutagenesis with bisulphite has revealed

Table 1.2 Point mutations activating members of the *ras* gene family in human and murine tumours

Tumour from which *ras* oncogene was identified	Amino acid in p21 protein product	Mutation	
Human bladder carcinoma	Position 12 in Ha-*ras*	GGC glycine	GTC valine
Rat mammary carcinoma	Position 12 in Ha-*ras*	GGA glycine	GAA glutamic acid
Human lung carcinoma	Position 61 in Ha-ras	CAG glutamine	CTG leucine
Human lung carcinoma	Position 12 in Ki-*ras*	GGT glycine	TGT cysteine
Human colon carcinoma	Position 12 in Ki-*ras*	GGT glycine	GTT valine
Human lung and bladder tumour	Position 12 in Ki-*ras*	GGT glycine	CGT arginine
Human neuroblastoma and fibrosarcoma	Position 61 in N-*ras*	CAA *glutamine*	AAA *lysine*

other amino acids in the human c-Ha-*ras*-1 gene where amino acid substitutions by mutation lead to a gene with transforming activity. However, these other sites are all clustered around amino acid 12 or amino acid 61. Substitution at amino acids 13, 59 and 63 leads to transforming activity. Some of these alterations give rise to less potent transforming genes, so called weak alleles, which might not be detected in the transfection assay with uncloned genes (Fasano et al, 1984).

In most cases, no overall increase in expression of the *ras* genes were seen in the tumours when compared to normal tissue (Tabin et al, 1982; Hall et al, 1983). However, the expression of the activated *ras* sequences may be altered in some cases. Two tumours appear to be homozygous for the mutated alleles (Taparowsky et al, 1982; Capon et al, 1983) and another tumour, which is heterozygous at the *ras* locus, has a transcriptional bias towards the expression of the activated allele (Capon et al, 1983).

Interestingly, the transfection assay only detects activated *ras* genes in about 10% of the tumours tested. In the remaining 90% of tumours, some other gene may be changed whose activity may not be detectable by transfection experiments. Additionally, some tumours may contain *ras* genes whose activation, for example by gene amplification (Schwab et al, 1983), would not be detected by transfection assays. *Ras* genes have been found to be activated in a wide variety of tumours and therefore do not appear to be tissue or tumour specific. In contrast to this the transforming genes detected by G. Cooper's laboratory, are highly tumour specific and appear to be activated in almost every example of that tumour (Lane et al, 1981, 1982). These oncogenes do not appear to be related to viral oncogenes. The fact that only one family of c-*onc* homologous to the v-*oncs* has been implicated in human tumours by the transfection assay may simply reflect the inability of the 3T3 cells to become transformed by any other activated c-*oncs* in this assay, although many of the v-*oncs* are capable of transforming these cells. An alteration in the assay may make it more