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Multistage Carcinogenesis in Paediatric and Adult Cancers

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Retinoblastoma (RB) and the familial adenomatous polyposis/colorectal cancer (FAP/CRC) complex provide well-characterised examples of multistage carcinogenesis and inheritance of a predisposition to cancer. Retinoblastoma appears to conform to the simple two-step model first proposed by Knudson. The gene responsible for RB, now called Rb1, has been located in chromosome region 13q14. The Rb1 gene has been cloned and subjected to extensive analysis. It is probable that the Rb1 gene product has a role in the regulation of transcription. The familial form of RB occurs as the result of a germline mutation of one of the copies of the Rb1 gene. Colorectal cancer, in contrast, appears to be the result of four or five steps involving both activation of oncogenes and inactivation of antioncogenes. The FAP gene has been located in chromosome region 5q21 by genetic linkage, and a candidate gene, MCC (mutated in colon cancer), has been cloned. Other mutations in previously-identified genes that have been identified as important in the genesis of CRC include the activation of p53 and of Ki-ras. A gene lying in chromosome region 18q which is deleted in colorectal cancer, and hence named DCC has been cloned. Its protein product has sequence homology to neural cell adhesion molecules and other related cell-surface glycoproteins. Delineation of the genes involved in the development of tumours such as RB and CRC provides insight into the mechanisms by which sequential mutations result in carcinogenesis.
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INTRODUCTION

THE CLONAL nature of cancer implies that malignancy must originate in a single cell. Both biological and epidemiological evidence suggests that the malignancy develops as a result of a number of discrete steps or mutations, which must, therefore, be accumulated in a single cell. The precise number of mutations required for the initiation of malignancy is variable, and probably depends on the tumour type and whether it is sporadic or familial. Retinoblastoma (RB) and familial adenomatous polyposis/colorectal cancer (FAP/CRC) provide well characterised examples of, respectively, simple and complex multistep carcinogenesis.

The occurrence of familial forms of various cancers implies the transmission of specific germline mutations. Individuals inheriting one such mutation are predisposed to a particular cancer, by virtue of inheriting one of the required initiating mutations. Once malignancy has been initiated, subsequent mutations may give rise to subclone with additional growth advantage; it is therefore important to distinguish between mutations necessary for initiation and those associated wtih progression.

RETINOBLASTOMA

Retinoblastoma, though rare, is the commonest intraocular cancer in childhood. About one-third of cases are familial, with the trait being transmitted as an autosomal dominant with very high penetrance [1]. In 1971, on the basis of a mathematical analysis of the ages at diagnosis of sporadic and familial RB patients, Knudson suggested that RB could arise as the result of as few as two mutations [2]. Subsequently, in 1978, he suggested that the two mutations could be the inactivation of both copies of an RB gene, each gene lying on a homologous chromosome [3].

The location of the RB gene has been defined in three ways: (a) the existence, in about 5% of patients, of cytologically visible chromosome deletions of chromosome 13 with a minimum region of overlap in the q14 region [4]; (b) genetic linkage, in affected families, between the RB phenotype and a protein polymorphism of the enzyme esterase D (ESD), the gene for which also lies in chromosome region 13q14 [5]; (c) the development of loss of heterozygosity for loci on chromosome 13, again with a minimum region of overlap in the q14 region, in sporadic tumours [6, 7].

Based on these data, a search was made for a gene expressed in fetal retinal cells but inactivated in RB tumours, culminating in the cloning of a candidate gene called 4.7R, by Friend *et al.* [8]. Subsequent work by many groups has authenticated this candidate gene, now called Rb1. Among the evidence is the inactivation of Rb1 in all RB tumours studied, the occurrence of germline mutations affecting Rb1 in patients with the familial form of RB and the transmission of specific germline mutations of Rb1 through families affected by RB [9–13].

Evidence in favour of Knudson's two mutation hypothesis first came from studies of ESD expression in RB tumours [6]. Further support has come from studies of DNA markers on chromosome 13 [14–16] and Rb1 mutations in tumours arising sporadically or as the result of germline (inherited) mutations [9, 10, 13].

The protein product of Rb1, $p105^{RB}$, is one of the targets of the transforming oncogene E1A of adenovirus 2 [17]. E1A forms complexes with a number of target proteins within an infected cell, and this process appears to be critical in achieving transformation. Subsequently, interactions between $p105^{RB}$ and transforming proteins SV40 large T and herpes virus 16 E7 have been discovered [18, 19]. Thus it seems that a number of DNA virus oncoproteins mediate transformation by a common

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mechanism, inactivating the Rb1 gene by forming complexes with $p105^{RB}$.

FAMILIAL ADENOMATOUS POLYPOSIS AND COLORECTAL CANCER

FAP/CRC provides a well characterised example of an adult cancer predisposition syndrome. FAP is inherited as a dominant trait. During adolescence affected individuals develop a variable number—from a few hundred to over a thousand—adenomatous polyps in the large bowel. These polyps are likely to give rise to adenocarcinomas in adult life, and thus prophylactic removal of the colon in FAP-affected individuals is usual.

The gene responsible for the development of FAP was found to be located on the long arm of chromosome 5 by linkage studies [20] following the observation of a deletion of the long arm of chromosome 5 in an individual with FAP, mental retardation and multiple developmental abnormalities [21]. However, only about 20% of sporadic colonic tumours are found to lose heterozygosity for polymorphic markers on chromosome region 5q [22], indicating the importance of other genetic events in the genesis of colorectal carcinoma. The gene from the 5q region has recently been cloned and is now called MCC (mutated in colorectal cancer) [23]. The MCC protein product has a region of similarity to the G-protein-coupled m3 muscarinic acetylcholine receptor. Studies of the MCC gene in two CRCs revealed somatically acquired point mutations resulting in amino acid substitutions.

Subsequent work has indicated that loss of heterozygosity in CRC is most frequently observed in chromosome regions 17p and 18q [24]. The gene lying in chromosome region 17p is now known to be p53 [25]. This gene was originally thought to be a dominantly-transforming oncogene [26]. However, p53 only possesses an active transforming ability if mutations have occurred within the gene [27]. The gene lying in chromosome region 18q has now been cloned and named deleted in colon cancer (DCC) [28]. The DCC gene encodes a protein with sequence similarity to neural cell adhesion molecules and other related cell surface glycoproteins.

In addition to the genetic changes discussed above, two further alterations have been noted in CRC tumours. It has been noted that CRC tumours contain a high incidence of mutations at position 12 in the Ki-*ras* oncogene [29, 30]. In addition, a significant loss of methyl groups in DNA has been found to occur early in the genesis of colorectal tumours [31].

It is now possible to construct a model of CRC tumorigenesis that is considerably more complex than the model for retinoblastoma. The development of CRC is as the result of at least four to five separate mutations, each resulting in some growth advantage for the affected cell; fewer mutations suffice for the genesis of benign tumours. The various mutations include both activation of oncogenes and the inactivation of anti-oncogenes. Although there is a preferred sequence of mutations, it is the accumulation of changes that, eventually, results in carcinogenesis [32].

CONCLUSION

The insights provided by the study of genes involved in the genesis of tumours such as RB and CRC will be of interest to developmental biologists. In addition to such basic biological information, knowledge of such genes will have clinical implications, allowing identification of mutant gene carriers, improving diagnostic precision and ultimately, perhaps, leading to novel forms of treatment.

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Note added in proof—Since this article was written a further gene in the chromosome 5q21 region has been cloned. This gene, named APC (for adenomatous polyposis coli) is situated in the same region as MCC. Individuals with germ-line mutations of APC develop colonic polyposis: in contrast no germ-line mutations of MCC have been found.

For review see Bourne HP, Suppression with a difference. *Nature* 1991, **353**, 696–697.

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Cytokine Modulation of Cell Growth and Role in Tumour Therapy

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Cytokines are a group of secreted proteins which act as intracellular signals co-ordinating the growth and function of cells in the haematopoietic systems. Despite often overlapping functions they appear to have evolved separately but their receptors do share several features suggesting a common ancestor. Taking interleukin 2 (IL2) as an example we discuss the mechanisms involved with the regulation of IL2, the interleukin 2 receptor (IL2R) and their modes of action. Finally we discuss the various aspects of cytokines which allow their use as antitumour agents.

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INTRODUCTION

THE CYTOKINES are a group of secreted proteins which act as inter-cellular regulatory factors. Their major role is to coordinate the growth and function of cells in the haematopoietic system. Thus they exert effects on cells ranging from precursors to mature effector cells. The nomenclature of the cytokines is fragmented reflecting the historical context in which they were discovered. It is now apparent that the categories are rather artificial and that there is much functional crossover between the various groups, which include the interleukins (ILs), the interferons (IFNs), tumour necrosis factor (TNF) and lymphotoxin, the colony-stimulating factors (CSFs) and erythropoietin.

The cytokines share the general characteristics that they are all relatively low molecular weight (less than 80 kD) proteins and act in a paracrine or autocrine manner. Certain cytokines have overlapping sets of functions, for example the colony stimulating factors (CSF), granulocyte macrophage CSF (GM-CSF), granulocyte CSF (G-CSF), macrophage CSF (M-CSF) and IL3 can exert identical effects on some bone marrow progenitor cells.

Despite overlapping functional activities of cytokines, they share surprisingly little amino acid sequence homology. There is some organisational similarity between the IL6 and GCSF genes in terms of intron/exon structure suggesting a possible ancient relationship [1], but it appears that in general the cytokine genes are unrelated and have evolved separately.

There is, however, an interesting cluster of the genes for IL3, IL4, IL5, GM-CSF, M-CSF and the M-CSF receptor [2, 3], on the distal portion of the long arm of chromosome 5. Loss of either the whole, or the long arm, of chromosome 5 has been observed in the myeloid cells of patients with some myeloid leukaemias and myelodysplastic syndromes [4, 5]. Several of the cytokine genes, GMCSF, G-CSF, IL3, IL2, IL4 and IL5, have a 10 bp concensus sequences in the 5′ flanking region [6, 7]. This is a transcription factor binding site for NF-GM (nuclear factor for GMCSF) which probably plays a regulatory role in cytokine gene expression.

Although the cytokines are structurally unrelated, several components of their receptors have marked amino acid sequence homologies. The receptors for which the signalling complex structures have been best defined appear to be heterodimers. The interleukin-2 receptor (IL2R) has two membrane spanning chains of 55 and 75 kD, both of which have ligand binding sites and form a dimer to give a high affinity receptor [8]. Similarly the IL3R and GM-CSFR are also heterodimers. In this case the two receptors share a common chain which may explain some of their overlapping biological functions [9]. Components of the IL2 receptor, IL3 receptor, IL4 receptor, IL5 receptor, IL6 receptor, IL7 receptor, the erythropoietin receptor, G-CSF

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