Urological and Genital Cancer

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Preface

The last decade has seen major changes in the management of both testicular and invasive bladder cancer as a consequence of the advent of durable complete remission using cisplatin-based combination chemotherapy. During this same time there have also been important improvements in the techniques of radiotherapy for treatment of invasive carcinoma of the bladder, prostate and penis, making it feasible to conserve the primary organ, although as with the breast those having radical surgery may have fewer painful local recurrences. In addition to the advances in chemotherapy and radiotherapy, there have been advances in hormone and immunotherapy. The development of gonadotrophin-releasing hormone analogues has provided a new option in the treatment of prostate cancer, while the use of genetic engineering to produce large quantities of interleukin-2 has provided a new treatment for renal cell carcinoma.

On top of all these clinical developments there have been major advances in our understanding of the basic science and epidemiology of cancer, most notably following upon the AIDS epidemic and our increased understanding of the molecular basis of cancer development following the discovery of oncogenes. Of profound importance has been the concept that mutation and/or translocation of a series of oncogenes by carcinogens, viruses and radiation is the final common pathway of cancer development with serial somatic mutation leading, in a Darwinian-like fashion, to survival of the most malignant clone in susceptible patients with the poorest DNA repair and immune surveillance capacity. This is at once more elaborate and more elegant a concept than the theories of initiation and promotion which were the basic idea of cancer generation for the last half a century. In addition to the theoretical significance, these concepts are already changing the practical management of patients, e.g. the role of circumcision and genital hygiene in the prevention of genital tumours, active intervention to alter smoking habits after bladder and testis tumours have been diagnosed, and management of undescended testis.

This book, conceived as a result of a British Council postgraduate course, aims to explain these developments and examines modern attitudes to management. It provides the postgraduate and active practitioner with a sound basis to enable them to understand the new ideas and contribute to the next decade's development.

R.T.D.O.

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JOHN A. WYKE

Introduction

The management of cancer is an empirical process. Its development has been influenced by conservative factors (the success or failure of previous management) that interplay with novel approaches based on contemporary ideas about the nature of the disease. The lack of significant reductions in most cancer morbidity and mortality implies a failure of this empiricism and provides the justification for continued attempts to understand the biology of cancer. Such attempts fall into two broad areas. Investigations into the *causes* of cancer try to identify factors, extrinsic and intrinsic to the organisms, that increase the risk of neoplasia. Studies on the *mechanisms* of cancer aim to unravel the processes by which a tumour develops and becomes clinically apparent.

Causes

Advances in understanding cancer causation have come largely from epidemiological studies. Variations in the pattern of cancer incidence in different human and animal populations point to the importance of extrinsic carcinogens: chemical, physical or infectious (mainly viral) agents. In some domestic and experimental animals exposure to certain carcinogens will cause disease in a high proportion of animals in a relatively short period. This is very rarely the case with man. Most humans exposed to a carcinogen usually fail to develop a tumour and this brings into prominence the importance of the dose of carcinogen and the length of the period of exposure. The commonest human cancers show a logarithmic increase in incidence with age, a pattern interpreted as reflecting the need for multiple changes in the genesis of a clinical tumour (Day 1983), these changes requiring either exposure to several risk factors or repeated exposure to a single agent. This complexity is, moreover, compounded by the likely influence of intrinsic but poorly understood factors that may be of particular importance to a long-lived species like man: the ability to repair damage inflicted by environmental carcinogens and the ability to eliminate or regulate incipiently neoplastic cells.

Despite these intricacies, environmental risk factors have been identified for a variety of human neoplasias. These discoveries raise the possibility of avoiding chemical and physical carcinogens and adopting prophylactic measures against viral risk factors. Unfortunately, practices aimed at complete avoidance may conflict with ingrained social habits or political and economic interests (Cairns 1978), whilst viral vaccines require at present enormous outlays for uncertain returns (Wyke & Weiss 1984). For the time being we must be content with measures whose efficacy depends on a

correct interpretation of the pattern of cancer incidence. Thus, if the disease is of multifactorial aetiology then it is only necessary to eliminate one factor to reduce greatly its prevalence. Moreover, reducing the dose of a carcinogen and the period of exposure should have beneficial effects even if exposure is not completely eliminated. These measures, however, only partly tackle the problem and even their success now depends on expertise in public relations rather than the force of scientific arguments. It is ironic that some of the most signal successes of cancer research can provoke antipathy as much as gratitude. Cancer prevention remains a long-term aim, but for the foreseeable future advances in cancer diagnosis and therapy will be demanded. Improvements in these two areas will require an understanding of the mechanisms of tumour development.

Mechanisms

The changes in cell growth control that result in neoplasia and in cell behaviour that lead to malignancy, are clearly very complex. This daunting intricacy has forced researchers to adopt a reductionist approach, dividing the problem into a series of potentially answerable questions each of which may lean heavily on model experimental systems. The answers to these questions have appeared at uneven rates governed by the conceptual and technological limitations of different approaches, the outcome being a collection of observations and explanations that may have been internally consistent but were not readily reconciled with the discoveries in different disciplines. The major excitement in cancer research over the past ten years has been a series of findings that, for the first time, has revealed common ground between many different lines of investigation. We may have found the corner-stones to the whole edifice of neoplasia and on these we can now start to build a complete description of the process. The structure is little more than scaffolding at present, over-simplified and begging many important questions, but enough of the framework exists for predictions of its future use in cancer management. The scale of recent advances and the enormous extent of our remaining ignorance can be judged by examining a seminal concept in cancer biology, the importance of somatic mutation.

Somatic mutation

The hypothesis that cancer results from genetic mutations was proposed long ago (Boveri 1914) but it was given a firm basis by experimental pathologists in the middle decades of this century (Foulds 1969). They appreciated that the phenotypes of cancer cells evolved by a series of generally irreversible steps and that a single cell could give rise to a tumour when inoculated into an appropriate normal host animal. Later workers were able to show that many, if not all, cancers are monoclonal and all these findings supported the idea that cancer arose by a series of stable, heritable changes inherent to the cancer cell lineage. The discovery of DNA as the repository for the genetic code, and the realization that many chemical and physical carcinogens were also mutagens that damaged DNA, led to the working hypothesis that these intrinsic changes were somatic mutations. It followed from this that carcinogen-induced mutations served to increase cancer over a background (due to 'spontaneous' mutations), that host abnormalities favouring mutations should increase cancer risk and that the inheritance of mutations affecting any stage of the neoplastic process should predispose to cancer in the affected individual (Knudson 1985).

Even without further elaboration, this concept proved heuristically valuable, stimulating experiments with important implications for cancer management. The main thrust of experimentation examined how chemical and physical carcinogens interacted with DNA, with several questions in mind (see Chapter 2). Firstly, attempts were made to identify the important structural features of chemicals that rendered them carcinogenic, with the aim of

drawing up guidelines to predict the carcinogenicity of novel compounds. This approach was largely unsuccessful but a second gambit, the prediction of a chemical's carcinogenicity on the basis of its mutagenicity in bacterial or eukaryotic cell test systems, has been widely used. Thirdly, a great deal of attention has been paid to the mechanisms in man and other animals that repair DNA lesions induced by carcinogens and other mutagens. These studies have a double significance. Defects in DNA repair may favour the genesis of neoplasia by carcinogens. However, and ironically, the chemical or physical agents that induce mutations will also generate lethal lesions in DNA, a feature underlying their use in cancer chemotherapy or radiotherapy. Mechanisms similar to those that protect cells against carcinogenic change may also contribute to the evolution of tumour cells that are refractory to therapy.

All these studies treat DNA as a chemical and largely ignore its biological role in determining and perpetuating the phenotype of the organism. Thus, although they have practical significance, they ignore the central questions raised by the somatic mutation theory of cancer. What genes are the targets for these somatic mutations? What are the effects of the mutations: do they inactivate genes, do they de-repress silent genes or do they alter the functions of genes? What are the roles of the proteins encoded by the target genes and how do their alterations lead to neoplasia? Cancer researchers could ask these questions thirty years ago but, faced with a cell containing 50 000 or more genes organized in an unknown way among a vast excess of non-coding DNA, they could only grope blindly for an answer. The way out of this dilemma was to approach the question tangentially by studying tumour viruses.

Tumour virology

The pathogenesis of virus-induced cancer has been studied since the beginning of this century (Gross 1970), but the golden age of tumour virology as an experimental tool arose from quantitative work on the interactions of bacteriophages with their hosts in the 1940s. A decade later the availability of animal cell culture in monolayer enabled the transfer of the same philosophy to work on the cytopathic animal viruses and this was soon extended to some tumour viruses. The ability of the latter to induce foci of morphologically altered cells in tissue culture not only permitted viral quantitation but also stimulated controlled studies on the phenotypes of these transformed cells, since they were considered the *in vitro* counterpart of tumours.

Tumour virologists in the 1960s mainly studied small viruses, with only enough genetic material to encode three or four proteins. They reasoned that, since these viruses transformed cells, one of the viral genes was a 'cancer gene', an oncogene, encoding a transforming protein. If this gene and its product could be discovered it would serve as a Trojan horse to gain access to the mysterious intracellular processes of neoplasia. We now realize that tumour viruses can initiate or promote neoplasia by a variety of means and many of the clinically more important do not contain an oncogene in their genome. However, it transpired that the small viruses that readily transform cells in culture, and were hence the most intensively studied in the laboratory, do possess oncogenes (Tooze 1980; Weiss *et al.* 1984, 1985). Those carried by the polyomavirus genus of the papovavirus family, by the human T-lymphotropic retroviruses and by bovine leukaemia virus have functions important for virus replication as well as cell transformation. However, the oncogenes carried by most so-called acutely transforming retroviruses are irrelevant to the virus life cycle and serve only to transform various cell types in the host. Small viruses do not usually carry superfluous genetic material, so presumably these oncogenes are foreign genes whose presence is accidental. Retroviruses, like many other tumour viruses, can achieve a chronic symbiosis with the host in which the viral genome is inserted in the host chromosome. It

was therefore not too surprising when, ten years ago, the several retroviral oncogenes then known were found to be related to, and apparently descended from, DNA sequences present in host cells.

This finding did not cause a great deal of excitement among cancer researchers in general, but its implications were not lost on tumour virologists (Bishop 1983, 1985). Retroviruses can apparently acquire host cell genes which are then responsible for virus-induced neoplasia. Could these cellular sequences, known as proto-oncogenes, be implicated in other neoplastic diseases? If so, then the study of tumour viruses has not only provided a model for investigating neoplasia but has also led directly to at least some of the elusive cell genes that are the targets for carcinogenic change.

Implicating proto-oncogenes in cancer

A great deal of evidence now suggests that proto-oncogenes can play a role in neoplasia. These indications, although circumstantial, are numerous and, as their ripples impinge in an ever widening circle on different aspects of cancer research, so studies in these separate disciplines further arraign proto-oncogenes. The incriminating data have been reviewed extensively in the past few years (Cooper 1982; Bishop 1983; Varmus 1984; Weiss *et al.* 1984, 1985) and we will not repeat them here, but will look at the evidence in two slightly different ways. Proto-oncogenes are normal cell genes, many of which have been strongly conserved through the evolution of eukaryotes. This implies important normal roles for these genes and presumably some alterations occur in their control or function in dysplastic cells. Somatic mutations in neoplasia have been shown to affect proto-oncogenes and the mutational alterations can be divided by pragmatic criteria into three categories (Table 1.1).

1 About five years ago several experienced tumour virologists reasoned that, since a viral oncogene acted in a dominant fashion to confer a neoplastic phenotype on a normal cell, then

Table 1.1 Mutations that may alter the products of known proto-oncogenes or perturb their expression*

Type of mutation	Implicating evidence	Examples
Point (single base change)	DNA transfection	Mutant c-H-*ras* or c-K-*ras*, detected sporadically or reproducibly in many tumours
Gross	Chromosomal translocation	c-*myc*, at chromosome 8 breakpoint in Burkitt's lymphoma, c-*abl* at chromosome 9 breakpoint in chronic granulocytic leukaemia
	Chromosomal amplification	c-*myc* in promyelocytic cell line, HL60; c-*erb* B in squamous cell carcinomas
Intermediate	Transduction by retroviruses	More than 20 viral oncogenes
	Insertional mutagenesis by virus and transposable elements	c-*myc* in chicken B-cell lymphomas, c-*erb* B in erythroblastosis
	Laboratory manipulation	c-*mos* and c-*ras* artificially linked to viral regulatory elements

*Original references are numerous. The interested reader should see reviews by Cooper (1982), Bishop (1983, 1985) and Varmus (1984) and in Weiss *et al.* (1984, 1985).

perhaps cellular genes altered in neoplasia may behave in a similar fashion. They therefore applied DNA from tumour cell lines to normal cells in culture ('transfection'), under conditions in which a minority of recipients incorporate the donor DNA into their own genome. DNA from certain tumour and transformed cell lines was shown to transform the recipients, a property later discovered in DNA isolated directly from some human tumours. It was then found that the transforming elements in the donor DNA were members of the c-*ras* family of proto-oncogenes (all oncogenes are identified by three letter sigla, the cell proto-oncogenes being prefixed by c-, their viral descendants by v-: the c-*ras* genes identified in tumours are ancestral to v-*ras* genes first described as the oncogenes of some rodent sarcoma viruses).

Why do c-*ras* genes from some tumours cause cell transformation whereas their counterparts, found in all normal cells, are inactive in this assay? The answer is that transforming *ras* genes all bear *point mutations* that change the amino acid sequence of their product at a limited number of sites in the protein. The specificity of these mutations can be remarkable. Some carcinogen-induced rodent tumours invariably contain 'activated' c-*ras* genes, all of which have an identical alteration in the amino acid sequence of their product.

2 The discovery of transforming c-*ras* genes that bear point mutations caused great interest among cancer researchers, but it was also appreciated that many cancer cells typically show more *gross mutations* that can be detected karyologically as chromosomal rearrangements. Among the best studied of these are chromosomal translocations or deletions, whose pattern can be very characteristic in certain tumours, and gene amplifications, often detected as double minute chromosomes or homogeneously staining regions on larger chromosomes. Known proto-oncogenes, such as c-*myc* and c-*abl* have been detected near the breakpoints of certain translocations, hinting that the translocations alter their activity. However, the effects of chromosome breaks on proto-oncogene structure and activity are variable. In some instances an unaltered gene product is expressed in an unregulated fashion, in other cases the coding region is altered and in further examples the mechanism of activation are not yet understood. Similarly, the consequences of proto-oncogene amplification in tumours, as can occur with the c-*myb* and c-*erb* B genes, await elucidation.

3 In view of the evidence implicating both point mutations and gross karyological alterations in cancer, it seems likely that other gene lesions may occur that are too insignificant to be detected karyologically, yet affect the integrity of a larger portion of the genome than would normally be transmitted in DNA transfer experiments. These *intermediate mutations* have received less attention than either point or gross mutations. This may be because they are truly less common, but it is intriguing that in many cancers there is no clear evidence for the importance of point or gross mutations, and lesions of intermediate magnitude may have been overlooked simply because we have fewer ready means to detect them. Indeed, the only good examples of this category of mutation have come from instances where we have a specific nucleic acid probe for the locus that is disrupted, as occurs when the DNA proviral form of a retrovirus inserts into a host cell chromosome. This integration of a provirus (and of anatomically similar cell transposable elements) is, of course, mutagenic since it alters the DNA sequence at the insertion site. Some of the earliest, and most compelling, evidence implicating proto-oncogenes in cancer came from studies on the pathogenesis of B-cell lymphomas of chickens, a tumour caused by a retrovirus that does not carry its own viral oncogene. Clonal tumours were found to contain a provirus integrated in the vicinity of, and affecting the activity of, the c-*myc* proto-oncogene and this motif of proviral insertional mutagenesis disrupting proto-oncogene activity is frequent in virus-induced tumours. Events of this type were probably the first steps in the

transduction of oncogenes by the acutely transforming retroviruses, the agents that first revealed these genes to us. In thymic lymphosarcomas of cats we even see a frequent recapitulation of this evolution; in many of these tumours the causative feline leukaemia virus has incorporated a copy of the cat c-*myc* gene into its own genome.

Looked at in a different way, the consequences of these mutations for proto-oncogene regulation and function can be manifold. Consider two genes, *A* and *B*, that are expressed at different levels (Fig. 1.1(a)) because they are regulated by control elements (hatched and solid boxes) of different potency. Let us suppose further that the activities of *A* and *B* are interdependent in some way, so that a relative increase in *B* function over *A* function will lead to neoplasia, an end that can be achieved in a number of ways. One set of mutations (Fig. 1.1(b)–(d)) do not change the level of *B* expression. Addition of a gene, *C*, that supersedes *B* function and is under the control of strong regulatory elements (Fig. 1.1(b)) is an intermediate mutation typified by integration of an oncogene-bearing tumour virus genome. Other intermediate mutations may reduce *A* function, by partially or completely deleting it (Fig. 1.1(c)), and if an effect depends on deleting both alleles of *A* such mutations would be recessive at the cellular level. On the other hand, *B* function can be directly altered, with-

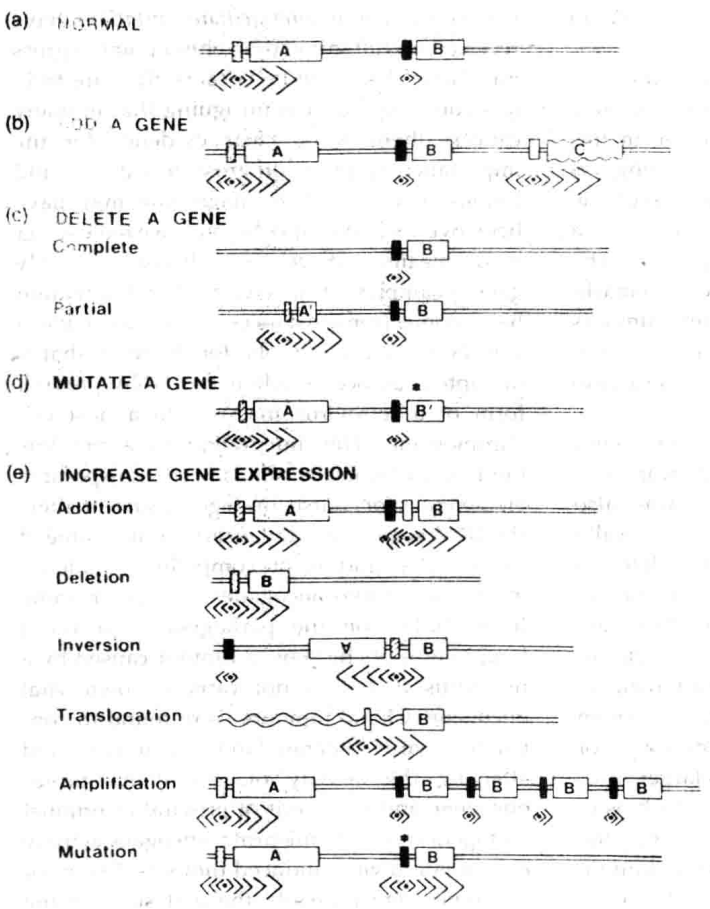

Fig. 1.1 Cancer: a genetic alteration. Open boxes A, B and C show coding regions of genes, A' and B' being mutated forms. Narrow boxes show regulatory regions of gene *A* (cross-hatched), gene *B* (solid) and an exogenous regulatory element (open). Chevrons below the regulatory regions depict the intensity and direction of influences increasing gene transcription. A star denotes a point mutation to a coding or regulatory region. For other explanations see text.

out changing its expression, by point mutations in its coding region (Fig. 1.1(d)), as occurs in *ras* gene activation. Another set of mutations alter the level of *B* expression (Fig. 1.1(e)). Addition of a powerful regulatory element can occur when a tumour virus acts as an insertional mutagen. Deletion or inversion of DNA sequences (mutations that can be gross or intermediate, depending on their extent) may place gene *B* under the influence of powerful cell regulatory elements and the same can occur in translocations. Note, however, that all these DNA rearrangements may affect the coding regions as well as the control regions of genes, leading to functional instead of regulatory mutations. The same proviso applies with gene amplification; extra copies may simply lead to an increase in *B* expression or they may include among them some with functional alterations. Finally, it is possible that simple point mutations to control regions may enhance *B* expression.

New oncogenes

We have noted examples in which gross, intermediate or point mutations affect the loci of proto-oncogenes, altering either the function or the regulation of these genes. It was possible to identify the genes at the mutated loci because their viral counterparts had already been characterized in the genomes of acutely transforming retroviruses. By extrapolation of this reasoning, it is argued that where a mutation of any category affects a locus that is not that of a known oncogene ancestor, then the region involved might encode a novel proto-oncogene. In this way, putative oncogenes have been identified as (i) transforming DNA in transfection assays; (ii) DNA sequences at chromosomal break points in translocations characteristic of certain tumours; (iii) sequences amplified in certain tumours; and (iv) sequences in the vicinity of tumour-specific integration sites of proviruses and other insertional mutagens. Examples of these candidate oncogenes, some of which are genetically related but not identical to known oncogenes, are given in Table 1.2. Since none of these genes have been naturally incorporated into retroviral genomes (where their biological effects are readily examined) their postulated role in neoplasia must now be confirmed by developing suitable assays for their functions.

Table 1.2 Detection of novel candidate oncogenes in man and animals*

Implicating evidence	Examples
DNA transfection	N-*ras* in neuroblastomas, sarcomas, lymphomas; *neu* in neuroblastomas, *mcf* 2 and 3 in mammary carcinoma
Chromosomal translocation	*bcr* in chronic granulocytic leukaemia, *bcl*-1 and -2 in chronic lymphocytic leukaemia
Gene amplification	*myc*-related genes in tumours of neurectodermal origin
Insertional mutagenesis	*int*-1 and -2 in mammary carcinoma, *pim*-1 in T-cell lymphoma, Mlvi-1 to -3 in T-cell lymphoma

*Original references are numerous. The interested reader should see reviews by Cooper (1982), Bishop (1983) and Varmus (1984) and in Weiss *et al.* (1984, 1985).