Advances in Urologic Oncology

Volume I

GENERAL PERSPECTIVES

Richard D. Williams

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Preface

Urologic oncology has become one of the most rapidly advancing areas of human tumor research. Recently, innovative techniques in diagnosis, such as computed tomography and radioimmunoassay, have considerably and irreversibly altered the approach to staging and thus treatment of urologic cancer. Currently newer modalities, such as magnetic resonance imaging and spectroscopy, flow cytometry, and monoclonal antibody methods, are beginning to provide sufficient information to be included in diagnostic evaluations of urologic cancer patients. Molecular biology has also begun to provide insight into the role of specific "cancer" genes in the development of urologic tumors. Effective variations of previous therapeutic modalities, such as hormonal therapy for prostate cancer, chemotherapy of uroepithelial cancers, and urinary diversion after surgical removal of the lower urinary tract, have only recently become available. Finally, laser applications, the use of immunotherapy, and endoscopic surgery of the upper urinary tract are evolving methods that have great promise but have yet to prove their specific efficacy in urologic cancer treatment.

In this book an attempt has been made to give comprehensive reviews of the most promising advances in the diagnosis and treatment of urologic cancer. As with any new and innovative technique only time will determine which of the perceived "advances" actually will prove to be clinically useful; yet it is expected that each of the areas described here will soon become

state-of-the-art methods used in the diagnosis or treatment of cancers of the urinary tract.

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1 Oncogene Expression and Chromosome Rearrangement in Urologic Tumors

Peter R. Carroll, M.D., William R. Fair, M.D., and R. S. K. Chaganti Ph.D.

INTRODUCTION

Boveri, in 1914, was one of the first scientists to propose that cancer was the result of a genetic event. Evidence for such a proposal comes from three sources (Bishop, 1985; Chaganti and Jhanwar, 1985). Patients with certain hereditary diseases, such as Beckwith–Weidemann syndrome, Fanconi's anemia, and familial polyposis coli, are more prone to develop certain tumors. Second, most tumors, when appropriately studied, will have abnormalities in chromosome structure or number. Finally, environmental exposure to mutagenic agents, such as radiation and certain chemicals, can damage genetic material and cause cancer. The primary neoplastic event is believed to be an alteration in deoxyribonucleic acid (DNA), the cell's informational molecule. In cancer, a cell's genetic structure is altered, allowing unrestrained cell division.

Deoxyribonucleic acid consists of two intertwined polynucleotide chains. Each chain is composed of alternating sugars, phosphate groups, and nitrogenous bases. The chains are held together by hydrogen bonds between the nitrogenous bases (Watson and Crick, 1953). The DNA is contained in chromosomes in the cell's nucleus. The base pair sequence of DNA contains the information necessary for cell proliferation, differentiation, and growth. During the process of transcription, DNA is transcribed into another nucleic acid, ribonucleic acid (RNA), which acts as a messenger and as a template

for the manufacture of proteins. The process of formation of a protein from the RNA template is called translation. A gene is a sequence of DNA that represents the information necessary for the manufacture of one protein. The human genome contains approximately 6 billion base pairs of DNA representing 1 million genes.

The molecular basis of cancer involves alteration in the normal transfer of information from DNA to RNA and ultimately to proteins. The ability to grow neoplastic cells in vitro; study individual chromosomes with high-resolution banding techniques; and isolate, identify, and clone certain genes and the proteins they encode has allowed meaningful insight into the molecular biology of cancer. Recently, attention has been directed to the identification of a class of genes called **cancer genes,** whose inappropriate activation may cause neoplastic transformation. At least one type of cancer gene are the **oncogenes** first identified in certain retroviruses (Bishop, 1983). The present chapter will review the structure, methods of activation, and encoded proteins of oncogenes. The significance of chromosomal rearrangements and oncogene expression in selected urologic tumors will then be discussed.

WHAT ARE ONCOGENES?

Peyton Rous, working at the Rockefeller Institute for Medical Research, injected a cell-free extract of chicken sarcomas into normal chickens and induced new sarcomas (Rous, 1911). Several years later, the agent responsible for tumor induction was discovered to be a virus, the Rous sarcoma virus (RSV), which belongs to a family of viruses called retroviruses because their genetic material is made up of RNA rather than DNA. They are called retroviruses because of their ability to transcribe their RNA into homologous DNA. (See Figure 1–1.) It was once thought that genetic information proceeded in only one direction: from DNA to RNA and finally to proteins. It was discovered that retroviruses possess an enzyme called **reverse transcriptase** that allows the viral RNA to be copied into homologous DNA. Once this viral DNA is incorporated into the host DNA, it directs the manufacture of viral proteins.

Some retroviruses induce neoplastic transformation in normal cells in culture and in susceptible animals. This process comes about through the integration of viral oncogenes into the host genome. The transforming gene in the RSV was labeled *v-src* for its origin in viruses and for the tumor (sarcoma) that it induces. A startling discovery was made when it was demonstrated, using molecular hybridization, that DNA homologous to the viral oncogene, *v-src,* was already present in all vertebrates from fish to humans (Stehelin et al., 1976). Subsequently, it was shown that retroviral oncogenes are actually copies of cellular genes picked up by viruses during viral infection sometime in the distant past (Bishop, 1981). These cellular homologues of *v-onc* genes are called cellular oncogenes or *c-onc* genes. Cellular oncogenes are ubiquitous throughout nature. Although not identical, there is striking similarity between cellular and viral oncogenes and the proteins these genes encode. Such evolutionary conservation suggests that these

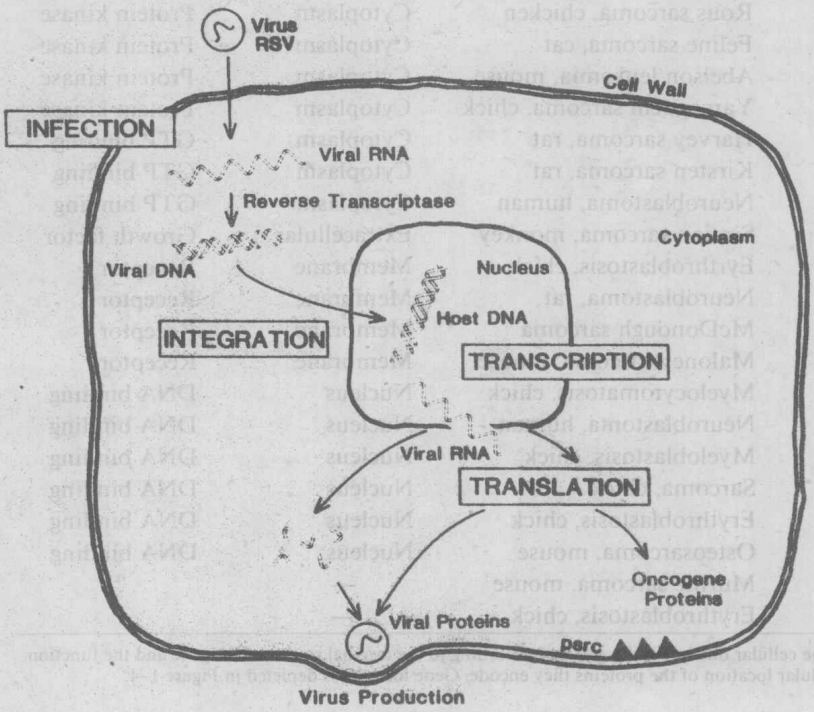

Figure 1–1. Infection of a cell by a retrovirus (see text).

genes may have additional roles in cells besides their association with tumor development. As we shall see, they may play important roles in normal cell growth and differentiation.

Almost 30 such oncogenes have been identified in retroviruses and in some tumors (Weinberg, 1982; Bishop, 1983; Gordon, 1985). (See Table 1–1.) A powerful tool for identifying *c-onc* genes has been transfection analysis. (See Figure 1–2.) DNA from a variety of sources, including human tumors, can be coprecipitated with calcium phosphate and applied to cultured cells. A small portion of the DNA is absorbed by the cells and integrated into the cell's DNA. The integrated DNA may be expressed in that cell and its descendents. The recipient cell line used almost exclusively in such experiments is a mouse fibroblast cell line designated NIH 3T3 (Shih et al., 1979). DNA from a variety of sources, including DNA complementary to retroviral RNA, human tumors, and normal cells treated with certain carcinogens have caused NIH 3T3 cell transformation. Transfection analysis, although useful, has been criticized for several reasons. The NIH 3T3 cells are an immortalized cell line and, therefore, may have already undergone partial progression toward a transformed state. In addition, only 20% of all cancers tested cause cell transformation using this assay (Duesberg, 1985).

Active cell proliferation is common to both early embryogenesis and neoplasia, which suggests that cellular oncogenes may play important roles in normal cell growth and differentiation. For instance, *N-myc* expression is elevated in normal mouse embryos at mid-gestation, but expression then decreases as term approaches (Jakobovits et al., 1985). A role for *N-myc* in

Table 1–1 Cellular Oncogenes*

Oncogene	*Origin*	*Cellular location*	*Function of protein*
src	Rous sarcoma, chicken	Cytoplasm	Protein kinase
fes	Feline sarcoma, cat	Cytoplasm	Protein kinase
abl	Abelson leukemia, mouse	Cytoplasm	Protein kinase
yes	Yamaguchi sarcoma, chick	Cytoplasm	Protein kinase
H-ras	Harvey sarcoma, rat	Cytoplasm	GTP binding
K-ras	Kirsten sarcoma, rat	Cytoplasm	GTP binding
N-ras	Neuroblastoma, human	Cytoplasm	GTP binding
sis	Simian sarcoma, monkey	Extracellular	Growth factor
erb-B	Eyrthroblastosis, chick	Membrane	Receptor
neu	Neuroblastoma, rat	Membrane	Receptor
fms	McDonough sarcoma	Membrane	Receptor
mos	Maloney sarcoma, mouse	Membrane	Receptor
myc	Myelocytomatosis, chick	Nucleus	DNA binding
N-myc	Neuroblastoma, human	Nucleus	DNA binding
myb	Myeloblastosis, chick	Nucleus	DNA binding
ski	Sarcoma, chick	Nucleus	DNA binding
ets	Erythroblastosis, chick	Nucleus	DNA binding
fos	Osteosarcoma, mouse	Nucleus	DNA binding
raf	Murine sarcoma, mouse	—	?
erb-A	Erythroblastosis, chick	—	?

* Many of the cellular oncogenes are grouped according to the original source of the gene and the function and subcellular location of the proteins they encode. Gene location is depicted in Figure 1–4.

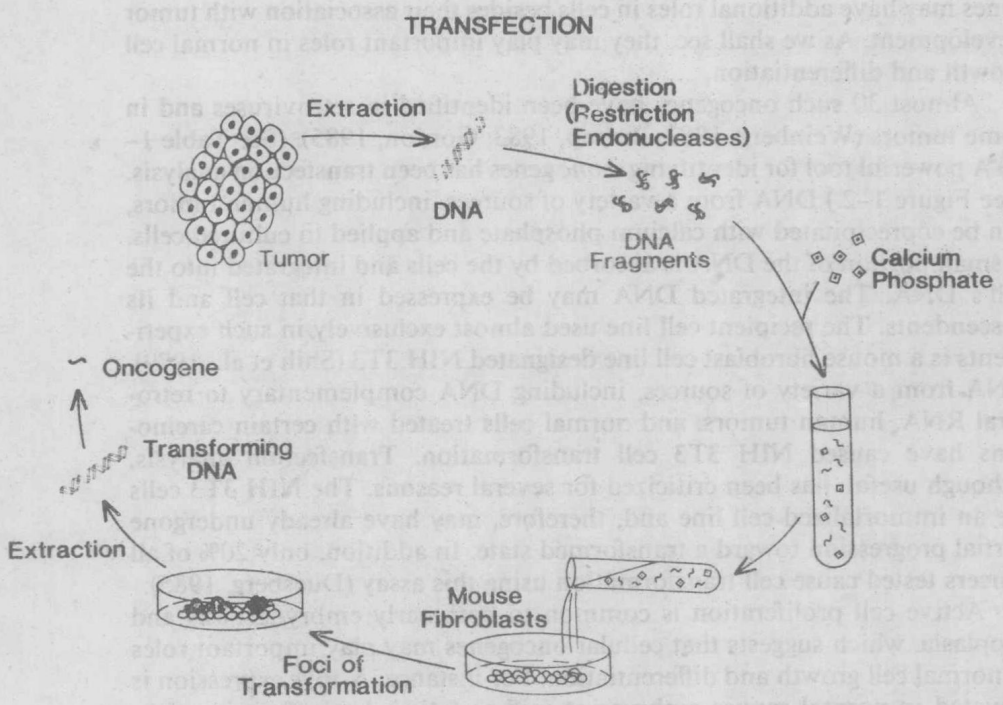

Figure 1–2. Transfection analysis (see text).

early stages of embryogenesis and cell growth also is supported by studies showing decreased expression of *N-myc* as certain cells are induced to undergo differentiation (Thiele et al., 1985). Slamon and Cline (1984) studied the expression of the cellular oncogenes *c-myc, c-erb, c-Ha-ras, c-src,* and *c-sis* during embryonic and fetal development of the mouse. Whereas *c-erb, c-myc,* and *c-src* genes showed increased expression consistently at certain times, the *c-Ha-ras* and *c-sis* genes were expressed at high levels throughout development. Other oncogenes, studied in a variety of tumors and cell lines, also show roles in differentiation and growth, (Gonda and Metcalf, 1984; Muller et al., 1985). Oncogene expression may also correlate with the stage of the cell cycle (Campisi et al., 1984). It seems reasonable to conclude that cellular oncogenes play important roles in normal cell growth. Cancer may be the product then of unrestrained or untimely oncogene expression.

As stated earlier, several retroviruses cause cancer in cell culture or in laboratory animals by integration of a *v-onc* gene into the host's genome. Although there is homology between *v-onc* and *c-onc* genes, is there any evidence to suggest that cellular oncogenes can cause cancer themselves? Support for such a concept comes from several sources. First, a certain retrovirus, the avian leukosis virus, lacks a recognizable oncogene, but does induce a variety of tumors. Analysis of DNA from induced bursal lymphomas in chickens revealed that a viral promotor gene is inserted next to the *c-myc* gene, which then becomes activated (Hayward et al., 1981; Payne et al., 1982). Second, two *c-onc* genes, *c-ras* and *c-mos*, when coupled to a retroviral promotor or enhancer induce transformation in NIH 3T3 cells (Blair et al., 1981). Third, several human tumor DNA samples induce cell transformation using the transfection assay (Shih et al., 1981; Cooper, 1982; Santos et al., 1982). The transforming genes in these experiments have been identified to be cellular oncogenes. Finally, an increased expression of several *c-onc* genes is seen in a variety of both solid and hemopoietic neoplasms (Slamon et al., 1984). It should be noted that more than one oncogene can be expressed in a given tumor. In fact, this may be the rule rather than the exception. Neoplastic transformation is generally considered to be a multistep process. Whereas a single, normal cellular oncogene is incapable of inducing cell transformation, a combination has been shown to be effective (Land et al., 1983).

The fact that oncogenes are part of a normal cell's genetic structure raises the possibility that the mechanisms needed for neoplastic growth are contained in all cells (Bishop, 1981). How cellular oncogenes become activated and how the proteins they encode cause cell transformation is the subject of the succeeding sections.

HOW ARE ONCOGENES ACTIVATED?

Presently, four mechanisms of oncogene activation have been described: activation by mutation, increased transcription due to promotor or enhancer sequences, amplification, and chromosomal rearrangement. In addition, it has been speculated that *c-onc* genes may become activated by loss of certain "suppressor" genes.

Mutation

It is well known that certain carcinogens, including radiation, chemicals, and even ultraviolet light, can cause cancer. They probably do this by damaging DNA. At the molecular level such damage, if not corrected by intrinsic cellular repair mechanisms, can have several consequences. A structural change in DNA can impair both replication and transcription. Changes in DNA's base pair sequence can occur as a result of base substitutions, deletions, insertions, or mispairings.

Mutation as a cause of oncogene activation has been studied by comparing the base pair sequence of normal cellular oncogenes and their retroviral and neoplastic counterparts. DNA is transcribed into RNA and is ultimately translated into proteins. Proteins are made up of amino acids. Three adjacent nucleotides or bases, called **codons**, code for one amino acid. A change in a single, critical nucleotide can lead to insertion of a different amino acid into an encoded protein. Such changes, if they occur at critical places, can have a profound effect on the proteins' structure and function.

The *ras* gene family has been studied extensively for the presence of base pair substitutions leading to oncogene activation. Although mutations probably occur at various sites within *ras* genes, it seems that oncogenic mutations occur at a restricted number of sites: codons 12, 13, 59, and 64 (Varmus, 1984). Single base pair substitutions in *ras* genes have been described for a number of human tumors and cell lines, including lung, bladder, and colon tumors and neuroblastoma and melanoma (Varmus, 1985). In each case, a single base change results in substitution of one amino acid for another. For instance, a substitution of thymidine for guanine in the 12th codon of the *c-Ha-ras* gene protein in the T24 bladder carcinoma cell line results in replacement of the amino acid glycine with valine. This change converts a normal cellular gene into an activated oncogene (Reddy et al., 1982; Capon et al., 1983). The same selective mutation sites have been confirmed in separate experiments by exposing the normal *ras* gene to random bisulfite mutagenesis. Only those *ras* genes with mutations at positions 12, 13, 59, or 63 possessed transforming activity (Fasano et al., 1984).

Point mutations can be studied by direct sequence analysis or more quickly by investigating restriction fragment length polymorphisms (RFLP). The latter is a method based on the finding that base pair changes in DNA can result in differential cutting of the sequence by certain enzymes (restriction endonucleases). Restriction endonucleases recognize a certain base sequence of DNA. If a sequence is modified by deletion, insertion, or substitution, changes in the number of restriction sites may occur. This is recognized as changes in the sizes of the strands of DNA once it is cut by the restriction endonuclease. Such changes can be recognized using Southern blot hybridization, a technique where DNA is separated on an electrophoretic gel, transferred to nitrocellulose, and hybridized with a radioactive probe that recognizes the *c-onc* gene sequence (Southern, 1975). Using restriction fragment polymorphisms, mutated, transforming *c-Ki-ras* and *c-Ha-ras* genes can be detected (Feinberg et al., 1983; Santos et al., 1984).

Cellular oncogene mutations are found in only a few human tumors (Duesberg, 1985). In addition, a mutated *c-onc* gene may have, by itself, limited powers. It can induce foci of transformation using 3T3 cells, but is unable to do so using many other cell types (Weinberg, 1982). It has been shown, however, that cooperation between oncogenes can induce cell transformation. If both *c-H-ras* and *c-myc* genes are applied to either rat-1 or rat embryo fibroblasts, transformation takes place. Neither oncogene by itself will cause transformation. These transformed cells will induce tumors in nude mice (Land et al., 1984).

Promotor Insertion

Oncogenes may be expressed at high levels due to the insertion of either viral promotor or enhancer sequences into host DNA in the same region as the cellular oncogene. Such an insertion seems to be the mechanism by which some retroviruses induce tumors only after long latent periods. A viral, transcriptional promotor (long terminal repeat) sequence is inserted next to a *c-onc* gene and causes the oncogene to be transcribed at a high rate. Although the protein product may not be altered, it is produced at unusually high levels and at an inappropriate time. Several oncogenes, including the *c-myc, c-Ha-ras,* and *c-myb* genes, seem to have been activated by such a mechanism in a variety of tumors (Land et al., 1984; Varmus, 1984).

Amplification

Increased gene dosage or copy number can lead to increased expression and ultimately increased production of an encoded protein. Such a mechanism is called amplification. Oncogene amplification has been described in several tumors and cell lines (Marx, 1983; Alt et al., 1985). In all instances, gene amplification is associated with increased production of homologous RNA. The chromosomes of such tumors frequently show the presence of double minute chromosomes (DM) or homogeneously staining regions (HSR). (See Figure 1–3.) Homogeneously staining regions are portions of chromosomes that are uniformly stained when treated with certain reagents. Double minutes are small, extrachromosomal elements that replicate autonomously. Both structures represent regions of gene amplification.

The *N-myc* gene has been shown to be amplified in neuroblastomas; the *c-myc* gene in small cell lung carcinoma, colon carcinoma, and myelocytic leukemia; the *c-abl* gene in a chronic myelogenous leukemia cell line; the *c-K-ras* gene in a murine adenocarcinoma; and the *c-erb* B gene in a human epidermoid carcinoma cell line (Varmus, 1984). As much as a 100- to 400-fold increase in oncogenes has been detected in some tumors and cell lines.

Amplification of genes encoding for certain enzymes has been established as a mechanism in which cancer cells gain resistance to certain chemotherapeutic agents (Schimke, 1982). Similarly, oncogene amplification may be a mechanism in which cancer cells may gain a growth advantage. Some support for this concept comes from studies of high-stage neuroblastomas and small cell lung carcinoma. Amplification of the *N-myc* gene has been associated with clinical Stages III and IV neuroblastoma (Brodeur et al.,