ADVANCES IN DNA SEQUENCE-SPECIFIC AGENTS

Series Editor: GRAHAM B. JONES

Volume Editor: MANLIO PALUMBO

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PREFACE

DNA sequence specificity plays a critical role in a number of biological processes, and influences a diverse range of molecular recognition phenomena, including protein–DNA, oligomer–DNA, and ligand–DNA interactions. This volume is intended to give the reader an up-to-date view of both the current status of, and developments to be expected in the near future, in research involving DNA interactive antitumor agents. In line with the intent of the series, special emphasis is thus placed on issues connected with sequence specificity and molecular recognition. Whereas volumes 1 and 2 were divided into subsections covering both analytical methods and applications, in this volume the entire focus is devoted to the macromolecule target specificity of DNA interactive developmental therapeutic agents of current interest.

A brief introduction to DNA interactive anticancer agents is included for readers who may benefit from an overview surrounding the developments that have contributed to our general understanding of this field. The following nine chapters have been carefully chosen so that they describe topics which are at the forefront of development in DNA-targeted cancer chemotherapy. Issues that have been addressed include the mechanisms of selective DNA topoisomerase I and II poisoning by antitumor agents (Chapters 1 and 2), sequence-specific recognition of DNA by groove-binding drugs and drug-conjugates (Chapters 3 and 4), recent developments in nitrogen mustard alkylating agents and their potential use for antibody-directed enzyme–prodrug therapy (Chapter 5), nonclassical platinum

anticancer complexes, including dinuclear and *trans*-platinum derivatives (Chapter 6), DNA cleaving antitumor chromoproteins containing reactive enediyne moieties, which exhibit interesting free-radical chemistry along with selective targeting (Chapter 7), the potential of new sequence-specific antisense and antigene therapy in oncology (Chapter 8), and finally the conceivable chemotherapeutic use of mimetics of the DNA structure, obtained by substitution of the sugar–phosphate natural chain with a peptide backbone, the so-called peptide nucleic acids (Chapter 9). Important approaches being currently investigated for selective cancer treatment, such as gene therapy and immunochemotherapy, are not discussed in this volume since they fall beyond its scope.

Graham B. Jones
Series Editor

Manlio Palumbo
Volume Editor

INTRODUCTION TO DNA SEQUENCE-SPECIFIC AGENTS

Manlio Palumbo

I. NEED FOR SPECIFIC ANTICANCER DRUGS

Cancer is a genetic disease characterized by impaired regulation of cell proliferation and differentiation mechanisms. Normal cells contain specific genes, the proto-oncogenes, which encode proteins involved in biological processes, e.g. signal transduction and control of gene expression. These growth-promoting genes are counterbalanced by growth (tumor)-suppressing genes: the delicate equilibrium between activation and suppression allows a correct progression of the cell through its vital cycle. Mutations which potentiate proto-oncogene functions, turning them into oncogenes, induce uncontrolled growth in tumor cells. Similarly, DNA damage

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at the level of tumor suppressor genes lets cell proliferation proceed unconstrained, thus allowing development of malignancy. The hyperproliferative effects due to proto-oncogenes are generated in a number of ways in cancer cells. Proto-oncogenes may undergo point mutations in their sequence or they can be overexpressed as a result of amplification or translocation mechanisms. In the former case they produce "wrong" signal proteins, which impair the signal-transduction pathway; in the latter case the product of the oncogene differs only in the amount, not in the nature, from the product of the proto-oncogene found in normal cells.

These observations clearly indicate how difficult it is to find a selective target for cancer chemotherapy. Many of the drugs used today for treating cancer patients are in fact practically nonselective and exhibit severe toxicity to normal tissues. Another limiting aspect of the current pharmacological therapy of cancer is the onset of resistance phenomena, which in many cases reflects an acquired capacity of developing defense mechanisms against drug treatment (multidrug resistance). Finally, chemotherapy with the presently available drugs can be valid for certain types of tumors but inadequate for common solid tumors. Hence, the requirement for novel effective and specific antineoplastic agents is stringent and pressing, as witnessed by intense efforts by many industrial and academic research teams.

II. NUCLEIC ACID BINDING

Since DNA is the depository of genetic information and alterations in its expression are responsible for disease, the search for anticancer drugs directed against DNA, DNA processing enzymes, or their complexes has represented a logical and fruitful approach.

Indeed, of the compounds widely employed in clinical practice since the late 1940s or presently in advanced clinical trials, a vast majority interact with DNA or with enzyme–DNA conjugates (Table 1). Even though antimetabolites are listed as "other drugs" in Table 1, they are also related to nucleic acids as they interfere with enzymatic reactions involved in DNA or RNA synthesis.

The results of a very large number of investigations show that reversible binding to DNA, which may occur by intercalative or groove-binding mechanisms, is the initial important step of a cascade of chemical and biochemical events causing cell cytotoxicity. Subsequent to these reversible interactions, the DNA-damaging events generally occur. There are a number of ways DNA-directed anticancer drugs injure the nucleic acid. They can be reactive per se and generate damaging species such as free radicals or carbonium ions, which cleave or alkylate DNA, or they can mediate the transfer of a free radical to other species able to degrade the nucleic acid including oxygen or hydroxyl groups. A more elaborate mechanism involves the DNA cutting/rejoining enzymes of the topoisomerase family. In this case drugs interfere with an enzyme–DNA cleavable complex, thereby inducing DNA strand scission. The modes of DNA damage for clinically useful DNA binding anticancer agents are summarized in Table 2.

Table 1. Chronological Development of Anticancer Drugs

Drugs Interacting with DNA (or DNA-Enzyme Complex)	*Other Drugs*
1940s	
Nitrogen Mustard	
1950s	
Methanesulfonates	Methotrexate
Busulfan	Mercaptopurine
Chlorambucil	
Cyclophosphamide	
1960s	
Melphalan	Fluorouracil
Actinomycin D	Vinca Alkaloids
	Thioguanine
	Cytosine Arabinoside
1970s	
Bleomycin	
Mitomycin	
Doxorubicin	
Dacarbazine	
Nitrosoureas	
cis-Platin	
1980s	
Epipodophyllotoxins	
Mitoxantrone	Interferon
Carboplatin	
Ifosfamide	
1990s	
Camptothecins[a]	
Enediynes[a]	Taxol[a]

Note: [a]Drugs undergoing clinical trials.

During the past few years important advances have been made in understanding the basic requirements to promote effective interactions in the drug–DNA complex, and in characterizing its structural features at a molecular level. In addition, biochemical information has become available on ternary interactions involving drug, nucleic acid, and nucleic acid processing enzymes. These include DNA and RNA polymerase, and, most relevant to anticancer activity, DNA topoisomerases. Although the initial events of drug-mediated DNA damage are now relatively well understood, a wealth of knowledge is still missing to connect them to the subsequent

Table 2. Type of DNA Damage Generated by Clinically Useful Anticancer Drugs Interacting with DNA

Drug	*Type of DNA-Damage*
Busulfan Chlorambucil Cyclophosphamide Dacarbazine Ifosfamide Melphalan Methanesulfonates Mitomycin Nitrogen Mustard Nitrosoureas	Alkylation of base nitrogens
Bleomycin Enediynes	Free radical strand-cleavage
Carboplatin *cis*-Platin	Platinum coordinaiton to purine nitrogens
Actinomycin D	Topoisomerase II-mediated strand-cleavage Interference with DNA processing enzymes
Amsacrine Doxorubicin Epipodofillotoxins Mitoxantrone	Topoisomerase II-mediated strand-cleavage
Camptothecins	Topoisomerase I-mediated strand-cleavage

cytotoxic events, which finally lead to cell death. In addition, as more is learned about the cell cycle and proliferation, other nucleic acid-related targets for chemotherapy are emerging that will require the development of new therapeutic strategies. Among the most promising appears to be telomerase, as it prevents the shortening of telomers and is activated in cancer tissues, but not in somatic tissues.

III. THE SEARCH FOR SPECIFICITY

The development of antineoplastic agents possessing a selective and targeted action at the DNA level would represent a major advance in the pharmacological treatment of cancer. Sequence specificity should be considered at two different levels: short-range specificity, which is characteristic for low molecular weight ligands and allows preferential binding to 2–5 consecutive DNA base pairs; and long-range specificity, which may provide effective recognition of one DNA sequence in the