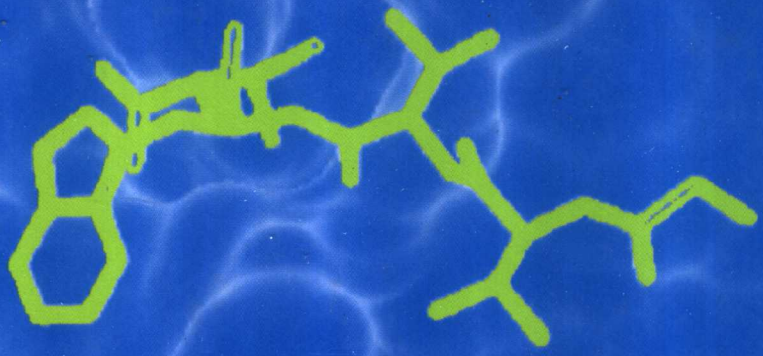
ANTICANCER DRUG DEVELOPMENT



EDITED BY

Bruce C. Baguley

David J. Kerr

ANTICANCER DRUG DEVELOPMENT

Edited by

Bruce C. Baguley

Auckland Cancer Society Research Centre

The University of Auckland

Auckland, New Zealand

David J. Kerr

Institute for Cancer Medicine

University of Oxford

Oxford, United Kingdom

ACADEMIC PRESS

A Division of Harcourt, Inc.

San Diego San Francisco New York Boston London Sydney Tokyo

This book is printed on acid-free paper.

Academic Press
A division of Harcourt, Inc.
525 B Street, Suite 1900, San Diego, California 92101-4495, USA
http://www.academicpress.com

Academic Press
Harcourt Place, 32 Jamestown Road, London NW1 7BY, UK
http://www.academicpress.com

Library of Congress Catalog Card Number: 2001090931

International Standard Book Number: 0-12-072651-3

PRINTED IN THE UNITED STATES OF AMERICA
01 02 03 04 05 06 EB 9 8 7 6 5 4 3 2 1

CONTRIBUTORS

Numbers in parentheses indicate page numbers on which authors' contributions begin.

Eric Ofori Aboagye (353) Cancer Research Campaign, Imperial College School of Medicine, Hammersmith Hospital, London W12 0NN, United Kingdom

Wynne Aherne (249) Cancer Research Campaign Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, United Kingdom

Bruce C. Baguley (1, 269) Auckland Cancer Society Research Centre, The University of Auckland, Auckland 1000, New Zealand

Alex Bridges (31) Pfizer Global Research and Development, Ann Arbor, Michigan 48105

Angelika M. Burger (285) Tumor Biology Centre, University of Freiburg, D-79110 Freiburg, Germany

Jian Cao (91) School of Medicine, State University of New York at Stony Brook, Stony Brook, New York 11794

Joseph M. Covey (301) Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Rockville, Maryland 20892

Thomas Davis (157) Oncology, Medarex, Annandale, New Jersey 08801

Stuart Decker (31) Pfizer Global Research and Development, Ann Arbor, Michigan 48105

Maja J. A. de Jonge (329) Department of Medical Oncology, Rotterdam Cancer Institute, 3075 EA Rotterdam, The Netherlands

William A. Denny (187) Auckland Cancer Society Research Centre, Faculty of Medical and Health Sciences, University of Auckland, Auckland 1000, New Zealand

Susan J. Donohue (301) Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Rockville, Maryland 20892

Nathalie Droin (55) INSERM U517, Faculty of Medicine, 21000 Dijon, France

Patrick Ducoroy (55) INSERM U517, Faculty of Medicine, 21000 Dijon, France

David Ferry (371) Department of Oncology, New Cross Hospital, Wolverhampton, West Midlands WV10 0QP, United Kingdom

Heinz-Herbert Fiebig (285) Tumor Biology Centre, University of Freiburg, D-79110 Freiburg, Germany

Rodolphe Filomenko (55) INSERM U517, Faculty of Medicine, 21000 Dijon, France

David W. Fry (31) Pfizer Global Research and Development, Ann Arbor, Michigan 48105

Michelle Garrett (249) Cancer Research Campaign Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, United Kingdom

Cherry L. Herald (203) Cancer Research Institute, Arizona State University, Tempe, Arizona 85287

Kevin O. Hicks (269) Auckland Cancer Society Research Centre, The University of Auckland, Auckland 1000, New Zealand

Fiona Hogan (203) Cancer Research Institute, Arizona State University, Tempe, Arizona 85287

Robert C. Jackson (171) Cyclacel Limited, Dundee DD1 5JJ, United Kingdom

David J. Kerr (371) Institute for Cancer Medicine, University of Oxford, 0X2 6HE, United Kingdom

Kurt W. Kohn (13) Laboratory of Molecular Pharmacology, Center for Cancer Research, National Institutes of Health, Bethesda, Maryland 20892

Elianne Koop (123) Laboratory of Medical Oncology, Division of Medical Oncology, University Medical Centre Utrecht, 3584 CX Utrecht, The Netherlands

Alan Kraker (31) Pfizer Global Research and Development, Ann Arbor, Michigan 48105

W. R. Leopold (31) Pfizer Global Research and Development, Ann Arbor, Michigan 48105

Walter J. Loos (329) Department of Medical Oncology, Rotterdam Cancer Institute, 3075 EA Rotterdam, The Netherlands

Ted McDonald (249) Cancer Research Campaign Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, United Kingdom

Christopher J. Molloy (91) 3-Dimensional Pharmaceuticals, Inc., Exton, Pennsylvania 19341

Ion Niculescu-Duvac (137) Cancer Research Campaign Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, United Kingdom

George R. Pettit (203) Cancer Research Institute, Arizona State University, Tempe, Arizona 85287

Stephanie Plenchette (55) INSERM U517, Faculty of Medicine, 21000 Dijon, France

Yves Pommier (13) Laboratory of Molecular Pharmacology, Center for Cancer Research, National Institutes of Health, Bethesda, Maryland 20892

Patricia M. Price (353) Cancer Research Campaign, Imperial College School of Medicine, Hammersmith Hospital, London W12 0NN, United Kingdom

Cédric Rebe (55) INSERM U517, Faculty of Medicine, 21000 Dijon, France

Julie K. Rhie (301) Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Rockville, Maryland 20892

Azeem Saleem (353) Cancer Research Campaign, Imperial College School of Medicine, Hammersmith Hospital, London W12 0NN, United Kingdom

Karen M. Schweikart (301) Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Rockville, Maryland 20892

Judith Sebolt-Leopold (31) Pfizer Global Research and Development, Ann Arbor, Michigan 48105

Sachdev Sidhu (237) Department of Protein Engineering, Genentech, Inc., South San Francisco, California 94080

Adaline C. Smith (301) Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Rockville, Maryland 20892

Eric Solary (55) INSERM U517, Faculty of Medicine, 21000 Dijon, France; and Hematology Unit, CHU le Bocage, BP1542, 21034 Dijon, France

Olivier Sordet (55) INSERM U517, Faculty of Medicine, 21000 Dijon, France

Alex Sparreboom (329) Department of Medical Oncology, Rotterdam Cancer Institute, 3075 EA Rotterdam, The Netherlands

Caroline J. Springer (137) Cancer Research Campaign Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, United Kingdom

Mario Sznol (157) Clinical Affairs, Vion Pharmaceuticals, New Haven, Connecticut 06511

Joseph Tomaszewski (301) Division of Cancer Treatment and Diagnosis, National Cancer Institute, National Institutes of Health, Rockville, Maryland 20892

Akihiro Tomida (77) Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan

Takashi Tsuruo (77) Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan

Jaap Verweij (329) Department of Medical Oncology, Rotterdam Cancer Institute, 3075 EA Rotterdam, The Netherlands

Emile E. Voest (123) Laboratory of Medical Oncology, Division of Medical Oncology, University Medical Centre Utrecht, 3584 CX Utrecht, The Netherlands

Gregory A. Weiss (237) Department of Protein Engineering, Genentech, Inc., South San Francisco, California 94080

William R. Wilson (269) Auckland Cancer Society Research Centre, The University of Auckland, Auckland 1000, New Zealand

Paul Workman (249) Cancer Research Campaign Centre for Cancer Therapeutics, Institute of Cancer Research, Sutton, Surrey SM2 5NG, United Kingdom

Anne Wotawa (55) INSERM U517, Faculty of Medicine, 21000 Dijon, France

Qiang Yu (13) Laboratory of Molecular Pharmacology, Center for Cancer Research, National Institutes of Health, Bethesda, Maryland 20892

Stanley Zucker (91) Department of Veterans Affairs Medical Center, Northport, New York 11768

PREFACE

The development of more effective drugs for treating patients with cancer has been a major human endeavor over the past 50 years, and the 21st century now promises some dramatic new directions. While improvements in surgery and radiotherapy have had a major impact on cancer treatment, the concept of systemic chemotherapy, specific for cancer cells and free of major side effects, remains a critical goal for the future. The issues underlying the achievement of this goal are complex, extending from an understanding of how cancer growth is controlled, through the technology of drug synthesis and testing, to the multifactorial requirements for clinical trial. For anyone working in any single area of anticancer drug development, it is important to have an overview of the whole process.

This book aims to provide such an overview. The opening chapters discuss possible targets for drug design, including the cell division cycle, growth signal transduction, apoptosis induction, and the manifold interactions between tumor cells and host tissues. Succeeding chapters then consider techniques of identifying new potential drugs, including molecular modeling, chemical synthesis, and screening. The concluding chapters detail the required steps that any new potential anticancer agent must go through before it can be considered for routine clinical treatment. In each of these areas, a number of eminently qualified contributors have provided commentaries. Inevitably there are areas of overlap, but these have been retained because they reflect the interdependence of different areas of research.

We hope that this book will provide a useful commentary, including both overviews and specific detail, on this vital but fascinating subject. We also hope that it will stimulate original thought and further encourage those from both scientific and medical backgrounds who are committed to improving the outlook of cancer patients worldwide.

Acknowledgments

This book represents the results of a considerable amount of work by many talented contributors. As editors we thank all of these contributors for their enthusiasm and patience. We thank our research colleagues for providing advice when needed and the staff of Academic Press for their support over many months. B.C.B particularly acknowledges the support of the Auckland Division of the Cancer Society of New Zealand, and through them the members of the public who have donated money for cancer research.

Bruce C. Baguley
David J. Kerr

CONTENTS

CHAPTER 10

STRUCTURE-BASED DRUG DESIGN AND ITS CONTRIBUTIONS TO CANCER CHEMOTHERAPY

Robert C. Jackson

CHAPTER 11

THE CONTRIBUTION OF SYNTHETIC ORGANIC CHEMISTRY TO ANTICANCER DRUG DEVELOPMENT

William A. Denny

CHAPTER 12

BIOSYNTHETIC PRODUCTS FOR ANTICANCER DRUG DESIGN AND TREATMENT: THE BRYOSTATINS

George R. Pettit, Cherry L. Herald, and Fiona Hogan

CHAPTER 13

DNA-ENCODED PEPTIDE LIBRARIES AND DRUG DISCOVERY

Sachdev S. Sidhu and Gregory A. Weiss

CHAPTER 14

MECHANISM-BASED HIGH-THROUGHPUT SCREENING FOR NOVEL ANTICANCER DRUG DISCOVERY

Wynne Aherne, Michelle Garrett, Ted McDonald, and Paul Workman

CHAPTER 15

TUMOR CELL CULTURES IN DRUG DEVELOPMENT

Bruce C. Baguley, Kevin O. Hicks, and William R. Wilson

CHAPTER 16

SCREENING USING ANIMAL SYSTEMS

Angelika M. Burger and Heinz-Herbert Fiebig

CHAPTER 17

RELEVANCE OF PRECLINICAL PHARMACOLOGY AND TOXICOLOGY TO PHASE I TRIAL EXTRAPOLATION TECHNIQUES: RELEVANCE OF ANIMAL TOXICOLOGY

Joseph E. Tomaszewski, Adaline C. Smith, Joseph M. Covey, Susan J. Donohue, Julie K. Rhie, and Karen M. Schweikart

CHAPTER 18

CLINICAL TRIAL DESIGN: INCORPORATION OF PHARMACOKINETIC, PHARMACODYNAMIC, AND PHARMACOGENETIC PRINCIPLES

Alex Sparreboom, Walter J. Loos, Maja J. A. de Jonge, and Jaap Verweij

CHAPTER 19

TUMOR IMAGING APPLICATIONS IN THE TESTING OF NEW DRUGS

Eric Ofori Aboagye, Azeem Saleem, and Patricia M. Price

CHAPTER 20

MECHANISTIC APPROACHES TO PHASE I CLINICAL TRIALS

David R. Ferry and David J. Kerr

CHAPTER 1

A BRIEF HISTORY OF CANCER CHEMOTHERAPY

Bruce C. Baguley
Auckland Cancer Society Research Centre
The University of Auckland
Auckland, New Zealand

Summary

Clinical cancer chemotherapy in the 20th century has been dominated by the development of genotoxic drugs, initiated by the discovery of the anticancer properties of nitrogen mustard and the folic acid analogue aminopterin in the 1940s. The development of inbred strains of mice in the early part of the 20th century led to the use of transplantable tumors for the screening of very large numbers of compounds, both natural and synthetic, for experimental antitumor activity. Such screening led to the identification of clinically useful drugs at a rate of approximately one every 2 years. New targets for cytotoxicity were identified in this program, including tubulin and DNA topoisomerases I and II. The huge expansion in our basic knowledge of cancer has facilitated the development of two new anticancer strategies: the inhibition of specific cellular growth pathways and the inhibition of growth of cancer as a tissue. One of the most important principles to emerge is that loss of growth control of cancer cells is mechanistically associated with an increased tendency to undergo programmed cell death, or apoptosis. Thus, cancer growth is a balance between cell birth and cell death. The balance is maintained not only by the genetic status of the cancer cell but by interactions with host cells and extracellular matrix components in the tumor environment. The identification of estrogen as a factor for stimulating the growth led to antiestrogens as therapeutic agents and, more recently, to antagonists of growth factor receptor-mediated pathways. The early use of bacterial toxins in cancer treatment has led to strategies based on host–tumor interactions, such as antiangiogenic and immune approaches. Current research has underlined the enormous complexity not only of growth and death control systems within the tumor cell but of interactions of tumor cells with vascular endothelial, immune, and other cells in cancer tissue. The challenge of future development of low-molecular-weight anticancer drugs is to apply knowledge gained in basic studies to develop new strategies.

1. Introduction

It is difficult to assign a date to the beginning of the treatment of cancer with drugs because herbal and other preparations have been used for cancer treatment since antiquity. However, the 1890s, a decade that represents an extraordinarily creative period in painting, music, literature, and technology, encompassed discoveries that were to set the scene for developments in cancer treatment in the 20th century. The discovery of penetrating radiation, or x-rays, by Roentgen in Germany in 1895 was complemented 3 years later by the discovery of radium by Marie and Pierre Curie. The discovery of ionizing radiation led not only to radiotherapy as form of cancer treatment but eventually to the development of anticancer drugs that mimicked the effect of radiation by

damaging DNA. The discovery by George Beatson, working in Scotland in 1896, that the growth of a breast cancer could be halted by removal of the ovaries indicated that the growth of cancer cells in the body could be influenced by external factors. This provided the basis for cancer treatment strategies that changed the regulation of cancer cell growth. The demonstration by William Coley in 1898 that the administration to cancer patients of a bacterial extract, sterilized by passage through a porcelain filter, caused regressions in lymphoma and sarcoma indicated that activation of the body's defense systems might provide a strategy for cancer treatment. Each of these three advances lent weight to the bold assumption, made by Paul Ehrlich and others in the early part of the 20th century, that low-molecular-weight drugs might be used in the management of cancer as well as infectious diseases. This chapter considers each of these three approaches in turn.

2. Genotoxic (Cytotoxic) Therapy

The first practical anticancer drugs were discovered accidentally. One such discovery was an outcome of war, stemming from the finding that sulfur mustard gas, used as a toxic vesicant in the First World War, caused myelosuppression. Although gas warfare was not employed in the Second World War, a considerable stock of mustard gas canisters was maintained in the Mediterranean area. An accident in the Italian port of Bari, involving leakage of one of these canisters, rekindled interest in the myelosuppressive effect of nitrogen mustard, leading to clinical trials in lymphoma patients (Karnofsky *et al.,* 1948; Kohn, 1996).

The identification of vitamins as small low-molecular-weight enzyme cofactors was an important biochemical achievement in the early part of the 20th century. The structural elucidation and crystallization of folic acid in 1946 led, as with other isolated vitamins, to studies on its effect on the course of a number of diseases. Unexpectedly, administration to leukemia patients of folic acid and its glutamylated derivatives resulted in an increase in tumor growth. While the use of low-folate diets in the management of leukemia was investigated, the development of the folic acid analogue aminopterin provided a significant advance in the management of childhood acute leukemia (Farber *et al.,* 1948; Bertino, 1979).

The link between these two disparate types of drugs and their biological activity was found to be related to their damaging effect on DNA. Although Friedrich Miescher had characterized DNA as a substance in 1862, the informational complexity and significance to life of DNA was not appreciated until the 1940s. The elucidation in 1953 by James Watson and Francis Crick of the double-helical structure of DNA had a singular impact on strategies of anticancer drug development. The cancer chemotherapeutic agent nitrogen mustard was found to react chemically with DNA (Kohn *et al.,* 1966). Studies on aminopterin indicated that it interrupted DNA biosynthesis and in so doing caused DNA damage. The next two decades brought a massive development of new drugs that affected the integrity of the cell's genetic material, with approximately one new drug entering widespread clinical use every 2 years. Many of these drugs, which revolutionized the treatment of many types of cancer, are shown in Figure 1.

A. Development of *in Vivo* Cancer Screening Systems

Developments in chromatography and analytical chemistry in the first half of the 20th century allowed compounds of defined structure to be isolated from a variety of plants, animals, and microorganisms (see Chapter 12). The evolution of synthetic organic chemistry over this time provided anticancer drugs in addition to antimicrobial and other medicinal drugs (see Chapter 11). It was quickly realized that it would be impossible to test such a large number of compounds in cancer patients and that some type of model tumor system was required. Transplantable animal cancers became accepted as the best basis for the screening of such drugs. This was made possible by the availability of inbred mouse strains, which had their beginnings in the early part of the 20th century. Three inbred strains of particular importance to anticancer drug screening—DBA, BALB/c, and C57BL—were introduced in 1909, 1916, and 1921, respectively. Spontaneous or carcinogen-induced tumors in these strains could be transplanted from one inbred mouse to another, allowing repeated testing of potential anticancer drugs (Stock, 1954). A detailed description of the role of animal testing in drug development is provided in Chapter 16. In the 1950s and 1960s, most testing programs used the transplantable L1210 and P388 murine leukemia models for primary screening and transplantable solid tumors for more advanced testing (Goldin *et al.,* 1981). The discovery of the athymic "nude" mouse, which had lost its ability to mount a cell-mediated immune response, allowed the testing of new drugs against human tumor material growing as xenografts in such mice (Rygaard and Povlsen, 1969).

B. Mitotic Poisons

Many of the early drugs that were screened for anticancer activity were derived from natural products. The plant product colchicine, isolated from the autumn crocus, was one of the first of these to demonstrate activity against experimental murine tumor models. It was found to induce arrest of cultured cells in mitosis and demonstrated a new mode of induction of genomic damage: that of disturbing the correct distribution of genetic material into daughter cells at mitosis. Colchicine, although useful at lower doses for the treatment

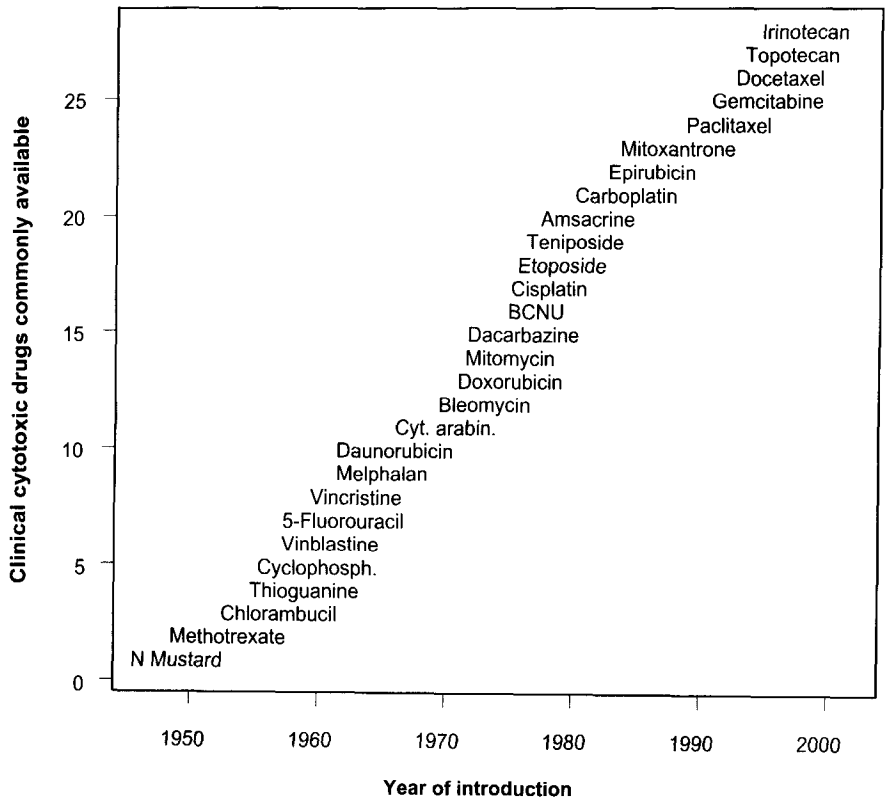


FIGURE 1 Chronology for the development of some of the anticancer drugs currently in use today. The abbreviations are N mustard (nitrogen mustard), cyt. arabin. (cytosine arabinoside), and BCNU (bischloroethylnitrosourea).

of gout, proved too toxic for use as an anticancer drug, and early attention focused on the *Vinca* alkaloids from the periwinkle plant (Johnson *et al.*, 1963). Two such alkaloids, vincristine and vinblastine, had a major impact on the early treatment of patients with malignancy (Rowinsky and Donehower, 1991). The protein tubulin was identified as the target for colchicine and the *Vinca* alkaloids (reviewed by Uppuluri *et al.*, 1993) and is the subject of considerable anticancer drug research. The broadening of the spectrum of tumor types susceptible to spindle poisons resulted from the discovery of the taxane class of compounds. Paclitaxel, discovered as a component of some *Taxus* species (Wani *et al.*, 1971), escaped detailed investigation until it was found to have a novel biochemical action distinct from that of the *Vinca* alkaloids, involving promotion rather than inhibition of microtubule assembly (Schiff *et al.*, 1979). Paclitaxel (Rowinsky *et al.*, 1990) and docetaxel (Bissery *et al.*, 1991) have a prominent place in cancer therapy today.

C. DNA-Reactive Drugs

Nitrogen mustard was the basis for the synthesis of a large series of clinically useful derivatives, including melphalan and cyclophosphamide, all found to exert their antitumor effects by alkylation of DNA. Natural products also yielded a number of clinically useful compounds that reacted chemically with DNA, such as mitomycin C (Whittington and Close, 1970), and bleomycin, which required the presence of oxygen and ferric ions to react (Crooke and Bradner, 1976). A particularly important development in DNA-reactive drugs came with the discovery of cisplatin, which had its origins in the chance observation that bacterial growth was inhibited around one of the platinum electrodes of an electrophoresis apparatus containing ammonium chloride in the buffer (Rosenberg *et al.*, 1965). Platination of DNA became a new mode of DNA damage induction and formed the basis for developing new analogues of cisplatin with reduced host toxicity.

D. Inhibitors of DNA Replication

A consequence of the elucidation of the structure of DNA was the rational design of analogues of the DNA bases, which were hypothesized to exert their anticancer activity by disruption of DNA replication. These included the thymine analogue 5-fluorouracil (Heidelberger *et al.*, 1957) and the purine analogues 6-mercaptopurine and 8-azaguanine (Hitchings and Elion, 1954). The cytotoxic effects of aminopterin and methotrexate were traced to their inhibition, through their

effect on the enzyme dihydrofolate reductase, of the conversion of deoxyuridine monophosphate to thymidine monophosphate. The phenomenon of "thymineless death," whereby bacteria unable to synthesize the DNA base thymine died in its absence, was found to have a parallel in mammalian cells and shaped the rationale for the development of the antimetabolite class of drugs. As the individual enzymes responsible for DNA replication were identified it became clear that the successful operation of the DNA replicase complex relied on a constant supply of the triphosphate precursors of DNA and that interruption of this supply resulted in damage to newly synthesized DNA. Natural products also played a role in the development of anticancer drugs acting on DNA replication. Arabinose nucleosides from the sponge *Cryptothethya* were found to have experimental antitumor activity, and one of them, the antimetabolite cytosine arabinoside, has found extensive use in the treatment of persons with leukemia (Ellison *et al.,* 1968). The testing of chemical analogues of cytosine arabinoside led more recently to drugs such as gemcitabine, which has activity against carcinoma (Plunkett *et al.,* 1996).

E. DNA Topology as a Target for Drug Development

In the 1960s, two logical problems concerning DNA structure became evident. The first was that the unwinding of DNA associated with DNA replication appeared to be thermodynamically impossible in the time frame involved, since the average chromosome would have tens of millions of helical twists. The second, familiar to anyone who has tried to untangle a fishing line, was that the separation of daughter DNA strands produced by DNA replication, prior to cell division, was thermodynamically impossible. Closed circular duplex DNA in some bacterial and mammalian viruses provided smaller molecular weight models for the study of these problems. Such DNA was found to exist in several distinct forms, each with the same sequence but with a different number of helical twists, and these were called topoisomers, based on topology (the branch of mathematics dealing with such differences in shape). In 1976, a new ATP-requiring enzyme, DNA gyrase, with the property of being able to change the topology of closed circular duplex DNA, was discovered in bacterial cells (Gellert *et al.,* 1976). DNA gyrase was found to have an essential role in DNA replication, and because it could pass one strand of DNA through another, it elegantly solved the problems of how DNA could be rapidly unwound during replication and how the daughter DNA strands could be separated after replication. Subsequent studies of mammalian cells demonstrated two main classes of enzymes. The first, topoisomerase I, changed DNA topology by breaking and rejoining a single DNA strand (Been and Champoux, 1980). The second, topoisomerase II, with some of the characteristics of bacterial gyrase, broke both strands of one double-stranded DNA to allow passage of a second double-helical strand through the breakage point (Miller *et al.,* 1981).

The discovery of the DNA topoisomerases also solved a problem concerning the activity of a number of natural products with anticancer activity that caused DNA damage but did not appear to react chemically. Actinomycin D, identified from *Streptomyces* cultures, found extensive early clinical use particularly in pediatric tumors (Farber *et al.,* 1960) and was found to bind DNA by intercalating its polycyclic chromophore between the base pairs of the DNA double helix (Müller and Crothers, 1968). It was of great biochemical interest because of its potent inhibition of RNA synthesis, but this did not appear to explain its antitumor activity. Subsequently, two anthracycline derivatives, daunorubicin and doxorubicin, were also found to bind DNA by intercalation of their chromophores, but their effects on RNA synthesis were less than those of actinomycin D. The clinical activity of daunorubicin was generally confined to hematologic malignancies but that of doxorubicin was broader (Arcamone, 1985). The synthetic compound amsacrine, which had clinical activity against acute leukemia (Arlin, 1989), bound DNA by intercalation of its acridine chromophore (Wilson *et al.,* 1981) but had little or no effect on RNA synthesis. Both amsacrine and doxorubicin were found to induce covalent links between DNA and proteins (Zwelling *et al.,* 1981), and subsequent work demonstrated that this protein was in fact the enzyme topoisomerase II (Nelson *et al.,* 1984). The drugs acted as poisons of this enzyme, subverting its normal function to one of inducing DNA damage.

A parallel development in plant natural product research provided podophyllotoxin analogues derived from the mandrake root (Stahelin and Von Wartburg, 1991). Podophyllotoxin itself, like colchicine, bound to tubulin, but some semisynthetic glycosidic derivatives, termed epipodophyllotoxins, were found to have superior experimental antitumor activity to podophyllotoxin itself. Etoposide, first tested clinically in 1971, was found to be useful against a variety of malignancies (Issell and Crooke, 1979). Investigation of the action of etoposide and of the related drug teniposide revealed that they had reduced binding to tubulin but induced DNA damage and poisoned the enzyme topoisomerase II. The plant product camptothecin, which did not bind DNA and previously had no known function, was found to be a specific poison for topoisomerase I (Hsiang *et al.,* 1985). Water-soluble analogues of camptothecin, such as topotecan and irinotecan, have clinical potential, and topoisomerase I is now an established tumor target (Pommier, 1993).

F. The Search for Selectivity

While the selectivity of radiotherapy was progressively increased by localization of the radiation field to specific areas

of tumor growth, the selectivity of cytotoxic therapy was dependent on particular properties of cancer tissue. The use of microbial models gave rise to the important concept that alkylating drugs killed cells in an exponential fashion, with a certain percentage of the cell population killed with each dose (Pittillo *et al.*, 1965). For some drugs, cytotoxicity was found to be maximal at a particular phase of the cell cycle. Skipper and colleagues (Skipper, 1967) used animal models to select administration schedules with optimal cytotoxicity for the cell-cycle-selective agents, and drug combination schedules that allowed optimal intensity of treatment. The spacing between treatments and the rate of appearance of resistant populations could make the difference between success and failure of treatment (Carl, 1989). Such reasoning was applied with success to hematologic malignancies, which often had a high rate of cell division, but was less successful in the management of solid tumors.

Another basis for selectivity was to exploit an enzyme or drug transport mechanism that was present to a different extent in tumor and normal cells. Many antimetabolites were found to exert their selectivity by such mechanisms. Topoisomerase enzymes provided a particularly good example of such selectivity since high cellular activity, which tended to occur in rapidly dividing cell populations, was associated with greater sensitivity to topoisomerase-directed anticancer drugs (Pommier, 1993). More recently, selectivity has been generated by the development of prodrugs, which have no cytotoxicity until an enzyme or other agent activates them. Initially, naturally occurring cellular enzymes, such as nitroreductases, were considered as candidates for activating prodrugs, but more recently the concept of introducing a different activating enzyme by means of a localizing antibody or gene therapy has been investigated. These concepts are discussed in Chapters 8 and 11.

3. Growth Control Pathways

While cytotoxic agents dominated the development of clinical cancer chemotherapy, the alternative approach of altering the signals that determine cancer growth was not forgotten. The demonstration by Beatson in 1896 of the role of the ovary in the progression of some types of breast cancer raised the question of whether the growth of all cancer types might be controlled by circulating hormones. Subsequent surgical studies showed that, apart from the case of prostate cancer, removal of endocrine glands was generally ineffective in cancer treatment. It was another 30 years before estrogen, one of the main hormones accounting for Beatson's result, was identified (Frank *et al.*, 1925), but the biochemical pathways linking steroid hormones to cell growth stimulation remained a mystery. During the 1960s and 1970s, studies of cultured cells, both normal and tumor, indicated that a diverse series of polypeptide growth factors were essential for cell growth, many specific for certain tissue types (reviewed by James and Bradshaw, 1984). An understanding of the action of such factors first required the elucidation of the molecular mechanism of regulation DNA replication and cell division.

A. The Cell Cycle Clock

One of the most fascinating questions posed by dividing normal and cancer cells was the nature of the molecular clock that instructed the cell as to when it would replicate its DNA and when it would divide. Early studies of cancer tissue identified mitotic cells by their morphology and DNA-synthesizing (S-phase) cells by their uptake of tritium-labeled thymidine, and these phases were found to be separated by periods of cell enlargement, termed G_1 and G_2 phase (reviewed by Tannock, 1978). The first clues to the nature of the oscillator that ran the molecular clock were provided by the discovery in developing sea urchin eggs of a protein termed *cyclin*. The cellular concentration of this protein increased up to the time of cell division and then abruptly decreased (Evans *et al.*, 1983). Studies of the division of fertilized frog eggs also indicated the presence of a cyclin, the synthesis of which was necessary to cell division (Cross *et al.*, 1989). A second component of the clock was identified from two lines of research, one using frog embryos and one using yeast mutants (Cross *et al.*, 1989). A specific enzyme, termed a cyclin-dependent kinase (cdk), was found both to associate physically with a cyclin and to be activated by it, providing a link between the oscillator and the actuator. Several distinct cdk's and cyclins were found to be present in mammalian cells. A third component of the clock mechanism was a protease (in the form of a proteasome) that was responsible for the degradation of the cyclin and thus the resetting of the clock (Glotzer *et al.*, 1991; King *et al.*, 1996). In the general scheme of the cell cycle (Fig. 2), the multiple functions required for cell division and DNA replication are exquisitely coordinated by cdk's 1 and 2, respectively, each activated at the appropriate time by specific associated cyclins. The cell cycle time for human tumors varies from around 2 days to several weeks (Wilson *et al.*, 1988).

B. Stopping and Starting the Cell Cycle Clock

While the majority of the body's cells are in a nondividing state, certain cells, such as blood cell precursors in the bone marrow and epithelial cells in the gut, are capable of dividing rapidly. Some mechanism must therefore regulate the passage of cells from a quiescent state to a dividing state. Most early studies utilized cultured fibroblasts to investigate this process. Time-lapse studies (Smith and Martin, 1973) indicated that the commitment to DNA replication and mitosis was determined by a stochastic (random) mechanism, and a

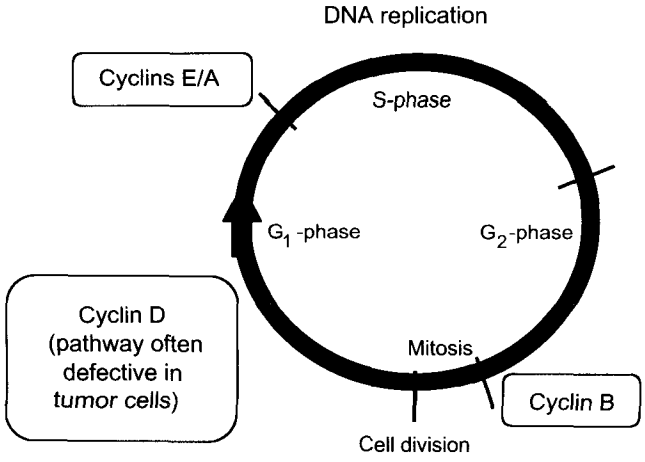


FIGURE 2 The cell cycle clock, with two key alternating processes: DNA replication and cell division. The timing of these processes is controlled primarily by oscillations (one per cell cycle) in the cellular levels of cyclin E/A and cyclin B, respectively. Activation of these processes is controlled by cyclin-dependent kinases 2 and 1, respectively. Normal cells have a further control on the decision to enter the cell cycle, timed by cyclin D and activated by cyclin-dependent kinases 4/6. This control system is deficient in cancer cells.

"restriction point" in the G_1 phase of the cell cycle was defined (Pardee, 1974), past which cells were irreversibly committed. A clear requirement was established for the presence of external polypeptide growth factors to allow passage of cells past the restriction point. Such factors were found to interact with membrane-bound surface receptors on target cells, and by the end of the 1970s it was established that at least some of these receptors, including that for epidermal growth factor, became phosphorylated as a consequence of growth factor engagement (Carpenter *et al.*, 1979). Internal cellular proteins were also phosphorylated in response to growth factors, but the identification of the complex linkages between growth factor receptors and commitment to DNA replication and cell division required new findings.

A major step forward in the identification of the pathway for initiation of cell growth had its origins in Peyton Rous's study of cancer-causing viruses in birds, mice, and rats in the early part of the 20th century (Rous, 1983). In 1983, the extraordinary finding was made that a gene in a tumor-transforming virus of simian apes was similar or identical to that specifying a known growth factor for cultured cells (Doolittle *et al.*, 1983; Waterfield *et al.*, 1983). Subsequent work defined a variety of genes that could be transmitted by retroviruses to the tissue of a variety of birds and animals, causing a tumorigenic change. As the functions of these corresponding gene products were elucidated, it was found that they mapped to biochemical pathways linking the binding of growth factors to the commitment of cells to DNA replication and cell division.

The above work led to identification not only of a network of regulatory proteins but also of new cyclins (D-cyclins) and cdk's (4 and 6) that control the passage of the cell from a quiescent phase into the cell cycle. The retinoblastoma protein and the transcription factors E2F and c-myc were also implicated in a complex control system that resulted in the up-regulation of the E and A cyclins and subsequent initiation of DNA replication.

As the elements of this control system were identified, it also became clear that the function of one or more of these elements was defective in cancer. For instance, many human cancers were found to be associated with a mutated *ras* oncogene such that the cells behaved as though they were being continuously stimulated by growth factors (Pronk and Bos, 1994). Furthermore, many cancer cells lacked the proper function of proteins, such as the retinoblastoma protein, that regulated entry into S phase (Herwig and Strauss, 1997).

C. Programmed Cell Death

In 1972, a pivotal hypothesis was advanced that cell death was, like progress through the cell cycle, a product of precise cellular programming (Kerr *et al.*, 1972). This hypothesis was to have a profound effect not only in explaining the loss of cells during the development of the embryo but in advancing our understanding of cancer growth. Apoptosis was found to be an energy-dependent process whereby a cell was converted to fragments that could be absorbed by surrounding tissue without the initiation of an inflammatory response (Wyllie, 1993). The molecular mechanisms of apoptosis are described in Chapter 4.

In a multicellular organism, loss of a single cell is generally unimportant. On the other hand, loss of growth control in a single cell could lead, in the absence of any protective mechanism, to unrestricted and catastrophic growth. The body appears to have a protective mechanism to ensure that any such cells losing growth control are eliminated. The mechanistic links are not yet fully defined, but transcription factors such as E2F and c-myc, which are involved in driving cells into the cell cycle, are also involved in driving cells into apoptosis (Evan and Littlewood, 1998; King and Cidlowski, 1998). Thus, cancer growth is a balance between cell birth and cell death, each initiated by the same pathway (Fig. 3). Cancer cells (as well as normal cells) can be prevented from undergoing apoptosis by so-called survival factors (Evan and Littlewood, 1998), such as insulin-like growth factor 1 (Juin *et al.*, 1999) and cell–matrix interactions (Meredith *et al.*, 1993).

D. The Cell Cycle Calendar

The pioneering studies of Hayflick showed that when human fibroblasts were cultured, they would die after a certain