Ann L. Jackman
Christopher P. Leamon *Editors*

Targeted Drug Strategies for Cancer and Inflammation

Springer

Targeted Drug Strategies for Cancer and Inflammation

Ann L. Jackman · Christopher P. Leamon
Editors

Targeted Drug Strategies for Cancer and Inflammation

Springer

Editors
Ann L. Jackman
Section of Medicine
Institute of Cancer Research
15 Cotswold Road, Sutton
Surrey SM2 5NG
UK
Ann.Jackman@icr.ac.uk

Christopher P. Leamon
Endocyte, Inc.
3000 Kent Avenue, Suite A1-100
West Lafayette, IN 47906
USA
Chrisleamon@Endocyte.com

ISBN 978-1-4419-8416-6 e-ISBN 978-1-4419-8417-3
DOI 10.1007/978-1-4419-8417-3
Springer New York Dordrecht Heidelberg London

Library of Congress Control Number: 2011928253

Preface

In 1999, a book entitled *Antifolate Drugs in Cancer Therapy* (Humana/Springer) focused on existing and emerging cancer drugs that inhibited folate-dependent enzymes. Several chapters in that volume provided evidence suggesting that the effectiveness and tolerability of antifolate therapy could be further increased by (a) understanding and exploiting some of the molecular determinants of drug sensitivity or (b) by reducing exposure of normal proliferating tissues to these agents. Now, a decade later, we can address the latter subject by reviewing the biological properties of a contemporary class of "targeted agents" that functionally exploit a tumor-associated folate transport protein called the folate receptor (FR).

The FR is a glycosylphosphatidyinositol-linked protein that captures its ligands from the extracellular milieu and transports them inside the cell via a nondestructive, recycling endosomal pathway. FRs have restricted expression in normal tissues, and they are not generally exposed to the bloodstream; however, elevated expression occurs in many human malignancies, especially when associated with aggressively growing cancers. These factors help define "FR targeting" as a viable tumor-targeting strategy. Agents that target the FR range in size from small molecule antifolate drugs and folate-drug conjugates to monoclonal antibodies and nanoparticles. In some cases, the agent need only bind to the FR to elicit a biochemical effect (e.g., diagnostic imaging or immunotherapy); in other cases, such as for high affinity antifolates and folate conjugates of small molecule therapeutics, internalization by the FR/endosomal apparatus and subsequent cytosolic delivery is required for biological activity against intracellular targets.

The discoveries highlighted in this book parallel the emergence of innovative "molecular targeted" small molecules and monoclonal antibodies, i.e., agents that target proteins within highly activated signal transduction pathways that control proliferation. However, many of the tumor-targeted strategies described within cross the boundaries between what is considered to be "molecular-targeted" vs. conventional systemic therapy. Obviously, for these novel agents to be effective, tumors must express a functional form of the FR. But in contrast to the targets of signaling inhibitors, tumor growth is not necessarily dependent on FR expression; rather, this cell surface receptor imparts key therapeutic specificity. Thus, while the

pharmacologic targets of FR-guided drugs and folate-drug conjugates are frequently those of conventional therapy, the selectivity realized through restricted tissue expression of the FR biomarker reduces the adverse effects against untargeted normal tissues. Regardless, both the cellular and molecular targeting approaches share the goal in shifting the paradigm from that of generalized chemotherapy to that of personalized medicine.

Beyond cancer research, FRs are also receiving attention from researchers of inflammatory disorders. Recent discoveries have shown that proinflammatory, activated human monocytes and macrophages express a functional FR isoform. Preclinical and clinical proof has already emerged showing how this marker can be used to identify sites of inflammation (e.g., arthritis) using folate-targeted radiodiagnostic imaging agents, and efforts for therapeutic exploitation are already underway (see Chaps. 9, 10). Clearly, it is only a matter of time before novel FR-targeted anti-inflammatory therapies reach clinical practice.

From a historical and complementary viewpoint, advances in our understanding of other folate transport proteins, such as the reduced folate carrier and the proton-coupled folate transporter, are also reviewed in this book (Chap. 1); however, the main theme of this volume is the FR, with much of the content focused on its basic biology and regulation (Chaps. 2, 3) as well as its exploitation for targeted therapy and diagnostic imaging (Chaps. 4–8). The contributors to this volume are all highly regarded in their fields, and we are very grateful to them for devoting so much time and effort into their excellent contributions. Both of us have benefited tremendously from reviewing their chapters, and we wish for their continued success.

Surrey, UK — Ann L. Jackman
West Lafayette, IN — Christopher P. Leamon

Contents

Contributors

Ndeye Diop-Bove
Departments of Molecular Pharmacology and Medicine,
Albert Einstein College of Medicine,
Bronx, NY 10461, USA

Silvana Canevari
Unit of Molecular Therapies, Department
of Experimental Oncology and Molecular Medicine,
Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Hala Elnakat
Department of Biochemistry and Cancer Biology,
University of Toledo College of Medicine,
3000 Arlington Avenue, Toledo, OH 43614, USA

Mariangela Figini
Unit of Molecular Therapies, Department
of Experimental Oncology and Molecular Medicine,
Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Aleem Gangjee
Graduate School of Pharmaceutical Sciences, Duquesne University,
600 Forbes Avenue, Pittsburgh, PA 15282, USA

I. David Goldman
Departments of Molecular Pharmacology and Medicine,
Albert Einstein College of Medicine,
Bronx, NY 10461, USA

Mesfin Gonit
Department of Biochemistry and Cancer Biology,
University of Toledo College of Medicine,
3000 Arlington Avenue, Toledo, OH 43614, USA

Michael J. Hansen
Department of Chemistry, Purdue University,
560 Oval Drive, West Lafayette, IN 47907, USA

Ann L. Jackman
Section of Medicine, Institute of Cancer Research,
15 Cotswold Road, Sutton, Surrey SM2 5NG, UK

Gerrit Jansen
Department of Rheumatology, VU Institute for Cancer and Immunology,
VU University Medical Center, de Boelelaan 1117,
1081 HV Amsterdam, The Netherlands

Barton A. Kamen
Cancer Institute of New Jersey, Robert Wood
Johnson Medical School, New Brunswick, NJ, USA

Christopher P. Leamon
Endocyte, Inc., 3000 Kent Avenue, Suite A1-100,
West Lafayette, IN 47906, USA

Philip S. Low
Department of Chemistry, Purdue University,
560 Oval Drive, West Lafayette, IN 47907, USA

Yingjuan Lu
Endocyte, Inc., 3000 Kent Avenue, Suite A1-100,
West Lafayette, IN 47906, USA

Larry H. Matherly
Development Therapeutics Program, Barbara Ann Karmanos Cancer Institute,
110 East Warren Avenue;
Department of Oncology; Department of Pharmacology,
Wayne State University School of Medicine, Detroit, MI 48201, USA

Cristina Müller
Center for Radiopharmaceutical Sciences ETH-PSI-USZ,
Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland

Matthew Ng
Section of Medicine, Institute of Cancer Research,
15 Cotswold Road, Sutton, Surrey SM2 5NG, UK

Manohar Ratnam
Department of Biochemistry and Cancer Biology,
University of Toledo College of Medicine,
3000 Arlington Avenue, Toledo, OH 43614, USA

Joseph A. Reddy
Endocyte, Inc., 3000 Kent Avenue, Suite A1-100,
West Lafayette, IN 47906-1075, USA

Marcela D'Alincourt Salazar
Department of Biochemistry and Cancer Biology,
University of Toledo College of Medicine,
3000 Arlington Avenue, Toledo, OH 43614, USA

Roger Schibli
Center for Radiopharmaceutical Sciences ETH-PSI-USZ,
Paul Scherrer Institute, 5232 Villigen-PSI, Switzerland;
Department of Chemistry and Applied Biosciences,
ETH Zurich, 8093 Zurich, Switzerland

Suneethi Sivakumaran
Department of Biochemistry and Cancer Biology,
University of Toledo College of Medicine,
3000 Arlington Avenue, Toledo, OH 43614, USA

Juan Zhang
Department of Biochemistry and Cancer Biology,
University of Toledo College of Medicine,
3000 Arlington Avenue, Toledo, OH 43614, USA

Chapter 1
Biological Role, Properties, and Therapeutic Applications of the Reduced Folate Carrier (RFC-SLC19A1) and the Proton-Coupled Folate Transporter (PCFT-SLC46A1)

Larry H. Matherly, Ndeye Diop-Bove, and I. David Goldman

Abstract The mechanisms by which folates are transported across cell membranes have been an area of research interest for nearly five decades. Major transport systems include the facilitative carriers, the reduced folate carrier (RFC) and the proton-coupled folate transporter (PCFT), and the high affinity folate receptors (FRs) α and β which transport folates by endocytosis. RFC is the major transport system in mammalian cells and tissues for folate cofactors and clinically relevant antifolate drugs including methotrexate, raltitrexed, pemetrexed, and pralatrexate. PCFT was identified in 2006 as the mechanism by which folates are transported across the apical brush border of the proximal small intestine. Whereas both PCFT and RFC are widely expressed in tumors, PCFT differs from RFC in its acidic pH optimum which favors transport at the low pH commonly found in the hypoxic microenvironment of solid tumors. Reflecting tumor-specific patterns of expression and/or function, recent studies have focused on the identification of folate-targeted therapeutics with selective transport by PCFT and FRs over RFC. The goal is to circumvent RFC and the potentially toxic consequences of drug transport by RFC in normal tissues. RFC in tumor cells can also influence the pharmacologic activity of PCFT and FR-selective agents by transporting physiological folates which compete for polyglutamylation and binding to intracellular targets. This review focuses on the facilitative pathways of (anti)folate transport, including RFC (SLC19A1) and PCFT (SLC46A1) in relation to their molecular properties, and their physiological and pharmacological roles.

L.H. Matherly (✉)
Developmental Therapeutics Program, Barbara Ann Karmanos Cancer Institute,
110 East Warren Avenue, Detroit, MI 48201, USA
and
Department of Oncology, Wayne State University School of Medicine,
Detroit, MI 48201, USA
and
Department of Pharmacology, Wayne State University School of Medicine,
Detroit, MI 48201, USA
e-mail: matherly@karmanos.org

A.L. Jackman and C.P. Leamon (eds.), *Targeted Drug Strategies for Cancer and Inflammation*, DOI 10.1007/978-1-4419-8417-3_1,

Keywords Proton-coupled folate transporter • Reduced folate carrier • Hereditary folate maladsorption • Antifolate • Folate

Abbreviations

AICAR	5-Amino-4-imidazolecarboxamide ribonucleotide
AICARTase	5-Amino-4-imidazolecarboxamide ribonucleotide formyltransferase
ALL	Acute lymphoblastic leukemia
BCRP	Breast-cancer resistant protein
CNS	Central nervous system
CSF	Cerebrospinal fluid
5-FormylTHF	5-Formyltetrahydrofolate
FR	Folate receptor
GARFTase	Glycinamide ribonucleotide formyltransferase
GlpT	Glycerol phosphate/inorganic phosphate antiporter
HFM	Hereditary folate malabsorption
LacY	Lactose/proton symporter
5-MethylTHF	5-Methyltetrahydrofolate
MFS	Major facilitator superfamily
MRP	Multidrug resistance-associated protein
mTOR	Mammalian target of rapamycin
MTX	Methotrexate
OAT	Organic anion transporters
PCFT	Proton-coupled folate transporter
RFC	Reduced folate carrier
RTX	Raltitrexed
SCAM	Substituted cysteine accessibility methods
THF	Tetrahydrofolate
TMD	Transmembrane domain
UTR	Untranslated region

1.1 Introduction

The mechanisms by which folates are transported across cell membranes have been an area of research interest for nearly five decades. Folate cofactors as vitamins are available only from exogenous sources. Reflecting this, there has been a long-standing interest in the mechanism by which these compounds are absorbed in the small intestine (Halsted 1979; Selhub and Rosenberg 1981; Said 2004). Studies on transport of antifolates date from mid- to late 1960s when it was recognized that membrane transport of methotrexate (MTX) is carrier-mediated and is an important determinant of MTX chemotherapeutic activity, and that tumor cells commonly develop resistance to MTX due to an acquired defect in cellular uptake (Sirotnak et al. 1968; Goldman et al. 1968; Hakala 1965).