River Publishers Series in Research and Business Chronicles: Biotechnology and Medicine





Post-genomic Approaches in Cancer and Nano Medicine

Kishore R Sakharkar

OmicsVista, Singapore

Meena K Sakharkar

Department of Pharmacy and Nutrition, University of Saskatchewan, SK, Canada

Ramesh Chandra

Department of Chemistry, Delhi University, India B. R. Ambedkar Center for Biomedical Research, University of Delhi, India





Published, sold and distributed by: River Publishers Niels Jernes Vej 10 9220 Aalborg Ø Denmark

ISBN: 978-87-93102-86-6 (Hardback) 978-87-93102-87-3 (Ebook)

©2015 River Publishers

All rights reserved. No part of this publication may be reproduced, stored in a retrieval system, or transmitted in any form or by any means, mechanical, photocopying, recording or otherwise, without prior written permission of the publishers.

Post-genomic Approaches in Cancer and Nano Medicine

RIVER PUBLISHERS SERIES IN RESEARCH AND BUSINESS CHRONICLES: BIOTECHNOLOGY AND MEDICINE

Volume 4

Series Editors

ALAIN VERTES

Sloan Fellow, NxR Biotechnologies, Basel, Switzerland

PAOLO DI NARDO

University of Rome Tor Vergata, Italy

PRANELA RAMESHWAR

Rutgers University, USA

Combining a deep and focused exploration of areas of basic and applied science with their fundamental business issues, the series highlights societal benefits, technical and business hurdles, and economic potentials of emerging and new technologies. In combination, the volumes relevant to a particular focus topic cluster analyses of key aspects of each of the elements of the corresponding value chain.

Aiming primarily at providing detailed snapshots of critical issues in biotechnology and medicine that are reaching a tipping point in financial investment or industrial deployment, the scope of the series encompasses various specialty areas including pharmaceutical sciences and healthcare, industrial biotechnology, and biomaterials. Areas of primary interest comprise immunology, virology, microbiology, molecular biology, stem cells, hematopoiesis, oncology, regenerative medicine, biologics, polymer science, formulation and drug delivery, renewable chemicals, manufacturing, and biorefineries.

Each volume presents comprehensive review and opinion articles covering all fundamental aspect of the focus topic. The editors/authors of each volume are experts in their respective fields and publications are peer-reviewed.

For a list of other books in this series, visit www.riverpublishers.com http://riverpublishers.com/series.php?msg=Research and Business Chronicles: Biotechnology and Medicine

Series Note

The deciphering in 2003 of the nucleotide sequence of the human genome, which followed the determination of the chromosomal sequences of an array of model microorganisms including Escherichia coli, Bacillus subtilis, and Saccharomyces cerevisiae, is a landmark in biology that has paved the way for a revolution in research and development in biotechnology and pharmaceutical sciences. Moreover, several novel discovery tools have since emerged, including dramatically enhanced computers, sequencing instruments with dramatically higher throughout and decreased costs, softwares conferring the ability to generate and manage very large amounts of data, and systems biology tools which have enabled in silico experiments and the creation of virtual patients or virtual microbes to both accelerate and increase the scope of pharmaceutical and biotechnological research. Whereas more than 10 years have already elapsed since this major scientific milestone, the translation into novel products, perhaps best exemplified by the current focus of pharmaceutical companies on personalised medicine, has only reached mainstream at the beginning of the present decade.

The impact of post-genomic approaches in cancer and nano-medicine development is the focal point of the present monograph. Starting with a review of underlying bases of cancer and the biology of coding and non-coding RNAs, principles of discovery of novel drugs including advances in animal models for oncology are laid out here. Revisiting the potential of natural compounds for the treatment and prevention of carcinomas, the discussion subsequently explores one of the next innovation S-curves in cancer therapeutics using nanomaterials as a case study. The ultimate purpose of the journey is to accelerate the development of disease-modifying pharmaceuticals, and answer unmet medical needs to enable cancer patients worldwide achieve remission and, ideally, cure.

Alain Vertès, Basel, Switzerland Pranela Rameshwar Rutgers, USA Paolo di Nardo, Roma, Italy

9				
			α	
	,			
				•

Preface

Cancer is a complex disease involving genomic alterations across several molecular mechanisms. Systematic and comprehensive elucidation of the molecular landscape of a wide range of cancers complemented by genome-wide approaches to interrogating the function of cancer genes and the vulnerabilities of tumors will pave the way for understanding the basic molecular mechanisms of cancer and applying this knowledge to transform the practice of cancer medicine. Alternative splicing has critical roles in normal cell function and development and can promote growth and survival in cancer. Aberrant splicing can lead to loss-of-function in tumor suppressors or activation of oncogenes and cancer pathways. Cancerspecific changes in splicing profiles can occur through mutations that are affecting splice sites and splicing control elements, and also by alteration in the expression of proteins that control splicing decisions. Chapter 1 presents a comprehensive review on alternative splicing and how it contributes to tumorigenesis by producing splice isoforms that can stimulate cell proliferation and cell migration or induce resistance to apoptosis and anticancer agents. Chapter 2 discusses the use of non-coding RNAs as molecular tools to understand the molecular mechanism of cell proliferation control during carcinogenesis, differentiation and drug-induced cytotoxicity. Malignant cells exhibit metabolic changes, when compared to their normal counterparts. Chapter 3 delineates the identification and validation of novel targets of nuclear hormone receptor (PPAR- γ) in glycolytic pathway and their role in breast cancer pathophysiology. Animal experiments have contributed significantly to our understanding of mechanisms of disease and mouse has been the model of choice. Chapter 4 describes various mouse/rat models for cancer and infectious diseases. Chapter 5 describes the use of natural compounds for hepatocellular carcinoma.

The advent of nanotechnology promises revolutionizing many fields including oncology, by proposing advanced systems for cancer treatment.

xviii Preface

Targeted drug delivery systems are among the most successful examples of nanotechnology. In the past few years, there has been significant momentum in the field of nanomedicine with the development of novel nanoparticles for the diagnosis and treatment of cancer. Their small size, large surface area-to-volume ratio, and surface characteristics enable them to have viable carrier for site specific delivery of vaccines, genes, drugs and other biomolecules in the body. They also have compatibility with different administration routes, which makes them highly attractive in many aspects of oncology and infectious diseases. Chapter 6 through Chapter 11 discuss the use of nanoparticles in cancer therapeutics. In putting together this book, we have tried to bring to table the contributions of various experts towards some key aspects in drug discovery with focus on cancer and naomedicine. As editors of this book, we are grateful to all the contributors who have made this book possible.

Acknowledgements

On behalf of all the authors, we would like to thank all our mentors, colleagues and friends who instilled in us the culture of science. Without support from them, we could not have written this book. The unconditional love and support from our families is gratefully acknowledged.

Finally, we would like to take this opportunity to acknowledge the services of the team of River Publishers and everyone who collaborated in producing this book.

Kishore R. Sakharkar

Meena K. Sakharkar

Ramesh Chandra

此为试读,需要完整PDF请访问: www.ertongbook.com

List of Figures

Figure 1.1	Most important splicing signals	2
Figure 1.2	Molecular diversity increases as information flows	_
	from genes to proteins	3
Figure 1.3	Types of alternative splicing events: (A) exon skip-	
	ping, (B) 3' alternative border, (C) 5' alternative	
	border, (D) intron retention, (E) dual-specific splice	
	site, (F) mutually exclusive exons and (G) exon	
	skipping of multiple adjacent exons	4
Figure 1.4	RNA-Seq mapping, as implemented by TopHat. (A)	
	Mapping of splice-sites boundaries. (B) Amount of	
	aligned reads (read depth)	5
Figure 1.5	CD44 isoforms associated to cancer	10
Figure 2.1	Schematic representation of action of ribozymes and	
1 18 0	miRNA	26
Figure 2.2	Schmatic representation of use of randomized	
g	ribozyme library for identification of genes involved	
	in muscle differentiation: adopted from Wadhwa	
	et al. [26]	30
Figure 2.3	Demonstration of ARF-Per19p interaction in mouse	
rigure 2.0	(p19ARF), but not in human (p14ARF) cells.	
	Pex19p specific ribozymes increased the activity of	
	p19ARF only as shown in G. Adopted from Wadhwa	
	et al. [39]	33
Figure 2.4	Use of randomized for identification of genes	33
rigure 2.4	involved in killing od cancer cells by Ashwagandha	
	leaf extract (i-Extract)and its pure phytochemical,	
		37
F: 0.5	Withanone. Adopted from Widodo <i>et al.</i> [46]	37
Figure 2.5	Use of mortalin staining as a reporter for induction	
	of senescence in cancer cells and hence the identi-	
	fication of anticancer siRNAs. Adopted from Gao	4.1
	et al. [65]	41
Figure 2.6	A representative flowchart of miRNA det-	
	ection. Three major approaches to detect miRNAs;	

	hybridization, RT-PCR and cellular imaging are	
	shown	43
Figure 2.7	Flowchart describing the modification of miRNAs	
	for amplification is shown. In order to amplify	
	miRNA pools from the total RNA, miRNAs can	
	be tagged with adaptors based on their chemical	
	characteristics as shown	44
Figure 2.8	Schematic representation of molecular spotter	
	probe. The probe is designed to be quenched in the	
	presence of precursor RNA. It emits fluorescence	
	only upon hybridization to the mature RNA	47
Figure 2.9	Schematic representation of the dual-color sensor	
8	vector system. Reporter system enables the real-	
	time, quantitative detection of miRNA in single cells	
	using dual color fluorescent proteins	48
Figure 2.10	Demonstration of targeting of p53 and p21 WAF1 by	
	miR-296. Adopted from Yoon et al [88]	52
Figure 3.1	PPAR γ activation mechanism. Upon ligand acti-	
	vation PPAR γ heterodimerizes with Retinoid X	
	Receptor (RXR) in nucleus and binds to PPRE	
	and/or PACM motifs in the promoter region and	
	modulates the expression of genes downstream. The	
	consensus PPRE site consists of a direct repeat	
	of the sequence AGGTCA separated by a sin-	
	gle/double nucleotide, which is designated as DR-1	
	site/DR-2 site and PACM site consist of 15 bp	
	consensus sequence, TTCATTTGGACATTG. The	
	PACM motifs are reported to be more common than	
	PPREs	62
Figure 3.2	Molecular targets of PPAR γ and pathways associ-	_
1.8	ated [Adapted from 27]	63
Figure 3.3	Genomic structure of the human PPAR gamma gene	
	(5' end) and PPAR γ mRNA splicing forms and	
	protein variants. There are seven isoforms of PPAR γ	
	with common exons 1–6	64
Figure 3.4	Transcriptional regulation of PPAR γ gene targets.	_
- Build Diff	A PPAR protein binds PPRE/ PACM motifs in	
	combination with retinoid X recentors (RXRs) upon	

	ligand activation. The two paired up proteins then regulate transcription of various genes e.g. PPAR γ upon activation is known to down regulate glycolytic genes - PGK1 and PKM2, pH regulator -NHE1, anti-oxidant enzyme - MnSOD in breast cancer cells	64
Figure 3.5	PPAR γ activation inhibits many malignancies	65
Figure 3.6	Metabolic targets of PPAR γ . Many glycolytic enzymes are over expressed in cancers. Glycolytic enzyme pyruvate kinase-muscle 2 (PKM2) is a key regulator of tumor metabolism which promotes tumor growth and Warburg effect by switching between its dimeric form the active one, which has higher affinity for substrate Phosphoenol pyruvate (PEP) to tetrameric form the inactive form, with	
Figure 3.7	lower affinity for substrate PEP and vice-versa. This switching behavior of PKM2 keeps a balance of activation of many pathways including, glycerol, serine/glycine, ether/ester phospholipid pyrimidine biosynthesis (in green) and oxidative metabolism for energy production, thereby promoting tumor growth and tumor cell proliferation	67
Figure 3.7	by PPAR γ in human breast cancer cell lines – MDA-MB-231 and MCF-7. The human breast cancer cells were exposed to 10 μ M of PPAR γ inhibitor GW9662 for 4 h followed by 5 μ M and 10 μ M of 15d-PGJ2 for 48 h at 37 °C. PPAR γ activation by 15d-PGJ2 down regulated the expression of glycolytic enzyme, PGK1 and PKM2 in breast cancer cell lines - MDA-MB-231 (7A and 7C) and MCF-7 (7B and 7D). Inhibiting the activation of PPAR γ by the PPAR γ inhibitor GW9662, did not affect the expression of PGK1 and PKM2, suggesting the transcriptional regulation of these glycolytic genes	
Figure 3.8	by PPAR γ [Adapted from 27] Apotosis and PPAR γ . Apoptosis was initiated in human breast cancer cells lines MDA-MB-231 and MCF-7 upon 15d-PGJ2 activation of PPAR γ .	68

Figure 3.9	The breast cancer cells were exposed to 5 μ M and 10 μ M of 15d-PGJ2 for 48 h at 37 °C. Caspase dependent apoptosis was confirmed in MDA-MB-231 and MCF-7 by expression studies for active caspase 8 (8A and 8B) and chromatin condensation as assessed by nuclear specific dye Hoechst (8C and 8D) [Adapted from 27]	69
	ligand, 15d-PGJ2 induced loss of mitochondrial potential in human breast cancer cell lines -MDA-MB-231 and MCF-7 as assessed by potential dependent dye, JC-1. In healthy/non-apoptotic cells, JC-1 exists as a monomer in the cytosol (green) and accumulates as J-aggregates in the active mitochondria, which appear red. In apoptotic cells, due to loss mitochondrial potential relatively lower dye J-aggregates accumulates in the mitochondria than cytoplasm where it is remains as a monomer. Control breast cancer cell lines had higher Red/Green flouresence ratio than 15d-PGJ2	
Figure 3.10	treated test cells, suggesting the loss of mito- chondrial potential by PPAR γ ligand, 15d-PGJ2 [Adapted from 27]	70
Figure 3.11	regulation of NHE1 expression was observed. A similar pattern was followed at transcriptional level, suggesting PPAR γ regulation of NHE1 in breast cancer cell lines [Adapted from 44] PPAR γ novel ligand, Hydroxy hydroquinone (HHQ) induces intracellular Reactive Oxygen Species (ROS) formation in human breast cancer	71

Figure 3.11	cell lines - MDA-MB-231 and MCF-7. The breast	
	cancer cells were treated with 12.5 μ M and 25 μ M	
	of HHQ for 48 h at 37 °C. Intracellular ROS for-	
	mation was found to be significantly increased in	
	HHQ treated cells as compared to control cells in a	
	dose dependent manner was reported. The effective	
	enhancement of ROS production by HHQ correlates	
	to its cytotoxicity nature	
	[Adapted from 52]	73
Figure 3.12	PPAR γ activation by 15d-PGJ2 represses MnSOD	
	mRNA and protein levels in human breast cancer	
	cell lines - MDA-MB-231 and MDA-MB-468. The	
	breast cancer cells were exposed to 3 μ M, 5 μ M and	
	10 μM of 15d-PGJ2 for 24 h at 37 °C. A significant	
	repression of MnSOD level was observed in dose-	
	dependent manner [Adapted from 44]	74
Figure 4.1	Retroviral vector method	92
Figure 4.2	DNA microinjection method	93
Figure 4.3	Anti cancer drug targets	95
Figure 4.4	The Cell cycle	96
Figure 4.5	Air pouch model	97
Figure 4.6	Urinary tract infection	100
Figure 5.1	Deregulation of JAK/STAT3 pathway in Hepato-	
	cellular carcinoma: Upregulation of IL-6, Muta-	
	tions in gp130, Methylation of SOCS has been	
	reported as plausible mechanisms for deregula-	
	tion of JAK/STAT3 pathway. IL6: Interleukin-6;	
	JAK:Janus Kinase-2; STAT3:Signal Transducer and	
	Activation of transcription 3; SOCS: Suppressor of	
	cytokine signaling	112
Figure 5.2	Deregulation of β -catenin in hepatocellular carci-	
	noma: Upregulation of FZD7, mutations in AXINS,	
	production of stable beta-catenin and increase in	
	cell-cell adhesion have been postulated as plausible	
	mechanisms for deregulation of beta-catenin path-	
	way in HCC. FZD7: Frizzled-7 protein; GSK3B:	
	Glycogen Synthase kinase 3 beta; TCF: Transcrip-	
	tion factor	114

Figure 5.3	Deregulation of NFkB in hepatocellular carcinoma:	
S	Elevated levels of IL-6 and TNF α have been postu-	
	lated in constitutive activation of NFkB, thereby,	
	deregulation in hepatocellular carcinoma due to	
	upregulation of pro-survival signals. NFkB:Nuclear	
	factor kappa-light chain-enhancer of activated B	
	cells; TNF: Tumor necrosis factor; IL-6: Interleukin-	
	6; IKK: $I\kappa B$ kinase	115
Figure 5.4	Deregulation of PI3K/Akt/mTOR pathway in hep-	
O	atocellular carcinoma: Elevated levels of Akt	
	phosphorylation, overexpression of phoshomTOR,	
	somatic mutations of PTEN have been postu-	
	lated to play a plausible role in deregulation of	
	PI3K/Akt/mTOR pathway in hepatocellular carci-	
	noma. PTEN: Phosphatase and tensin homolog;	
	PI3K: Phophoinositide-3-kinase; IGF: Insulin-	
	like growth factor; EGF: Epidermal growth	
	factor; PDGF: Platelet derived growth fac-	
	tor; PDK1: Phosphoinositide-dependent kinase-	
	1; PIP2: Phosphophatidylinositol 4, 5-biphosphate;	
	PIP3:Phosphophatidylinositol 3, 4, 5-triphosphate;	
	Akt: Protein kinase B	118
Figure 6.1	The XRD spectra of ZnO nanoparticles	184
Figure 6.2	(a) The SEM image of synthesized ZnO NPs. (b)	
	TEM images of ZnO NPs. (c) HR-TEM of ZnO NPs	
	(d) SAED pattern	185
Figure 6.3	XPS spectra of ZnO nanoparticles. (a, b, c repre-	
	sents the scan over wide range and magnified band	
	structure at Zn and O level)	186
Figure 6.4	UV-Visible spectrum of ZnO NPs	187
Figure 6.5	Time –Kill curve of <i>E.coli</i>	187
Figure 6.6	Time –Kill curve of K.pneumoniae	188
Figure 6.7	Time –Kill curve of <i>S.paucimobilis</i>	189
Figure 6.8	Time –Kill curve of <i>P.aeruginosa</i>	189
Figure 7.1	Chemical structures of redox polymers possess-	
	ing nitroxide radicals, PEG-b-PMNT and PEG-b-	201
E: = 0	PMOT, and a redox nanoparticle (RNP)	201
Figure 7.2	In vitro characterization of RNP ^N and RNP ^O (a)	
	Effect of pH on the light scattering intensities of	