Histone Deacetylase Inhibitors in Combinatorial Anticancer Therapy

Shabir Ahmad Ganai

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Shabir Ahmad Ganai
Division of Basic Sciences and Humanities
Faculty of Agriculture
Wadura Sopore, SKUAST-Kashmir, India

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I dedicate this book to my wife Fatima Akhter, to my son Mohammad Faiq Ganai, and to my daughter Hafizah Shabir. While the writing of this book was ongoing, I was not able to give them company.

Foreword

Apart from the recent novel developments in cancer therapy such as immune checkpoint inhibitors or targeted agents focusing on altered cancer signaling pathways, the important role of epigenetic changes for tumorigenesis and tumor development as well as the potential of targeting these epigenetic changes as an alternative antineoplastic approach is increasingly accepted. In this context, the term epigenetics does not only refer to concepts that can explain how very different cellular phenotypes can arise without changes in the sequence or amount of inherited DNA, paradigmatic for the development of an organism but also to mechanisms in the cell nucleus that establish stable states of gene expression. Though it is increasingly well understood how mechanisms interact with each other to produce complex phenomena such as cell differentiation, gene dose compensation, or genomic imprinting, the question of how epigenetic changes interact in the development of malignant diseases and how we can target these changes remains a scientifically challenging one.

In this context, Dr. Shabir Ahmad Ganai aggregates the current knowledge on one of the most promising epigenetic targets, namely histone deacetylases (HDACs), in his book.

Dr. Shabir Ahmad Ganai has formerly remained the Principal Investigator/Young Scientist in the Department of Biotechnology, University of Kashmir. Following a masters degree from Karnataka and earning his Ph.D from Tamil Nadu, he dedicated his scientific efforts on understanding the modulations of the nuclear geometry and post-translational modifications during treatment with structurally distinct HDAC inhibitors. Further, he focuses on designing target-selective HDAC inhibitors using various Bioinformatics tools. So, given his scientific focus and the research efforts of our group and the use of isoenzyme-specific HDAC inhibitors in the antineoplastic treatment of urothelial carcinoma, it was just a matter of time that we stumbled upon each other's work.

In his book "Histone Deacetylase Inhibitors in Combinatorial Anticancer Therapy," Dr. Shabir Ahmad Ganai now explores the role of tumor epigenetics and antineoplastic epigenetic therapy strategies with a special focus on histone deacetylases (HDACs) and HDAC inhibitors.

Following a concise overview of the concept of epigenetics in health and disease, the epigenetic "key players," with a special focus on HDACs, important for human cancer biology are introduced to the interested reader. Further, from a biochemical point of view, current anticancer strategies including the use of epigenetic modulators in general and HDAC inhibitors in particular are outlined. Equipped with this fundamental knowledge, the potential use of these inhibitors as novel therapeutic agents both as singlet agent and as combination partners for other anticancer agents is discussed. For the interested reader, also novel solutions regarding the design of isozyme-selective inhibitors to improve both therapeutic efficacy and toxicity profile of HDAC inhibitors are discussed.

Both the basic researcher as the clinician-scientist (as myself) will find in this interesting book a valuable source of knowledge regarding tumor epigenetics in general and HDAC inhibitors in cancer medicine in particular bridging the gap between "bench" and "bedside."

Günter Niegisch

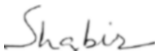
Department of Urology, Division of
Conservative Uro-Oncology,
Medical Faculty of the Heinrich-Heine-University
Düsseldorf, Germany

Preface

During my doctorate, I started working on small molecule inhibitors of histone deacetylases (HDACs). These inhibitors known as histone deacetylase inhibitors (HDACi) modulate gene expression programs following the epigenetic route. Through wet-lab experiments, I explored how structurally different HDACi sodium butyrate and entinostat alter the nuclear architecture and how they influence site-specific methylation in HeLa cells (cervical cancer cells) stably or transiently expressing H2B-EGFP and H3-EGFP and H3K9R-EGFP. These questions were addressed using **cell culture techniques, transient transfection, therapeutic intervention, mutagenesis (H3K9 to H3R9 conversion), immunofluorescence, and laser scanning confocal microscopy**. Following this, the second phase of my Ph.D. started with drug designing. In this phase, I worked on how minute structural differences at the active sites of Class II HDACs can be exploited for designing isozyme selective inhibitors. I used a variety of methods including **extra-precision molecular docking, molecular mechanics generalized born surface area (MMGBSA), and energetically optimized pharmacophores approach.** These methods were available in **Schrödinger Suite** which the university had purchased for the research purpose. In the third phase of my Ph.D., I worked on how HDAC inhibitor valproic acid in the human cell model alters site-specific methylation marks. A variety of techniques like **cell culture, pharmacological intervention, immunofluorescence, nuclear staining, quantitative PCR, confocal imaging, and molecular docking** were used for reaching a logical conclusion. As a **Principal Investigator/Young Scientist** at the **University of Kashmir (Department of Biotechnology)**, I worked on finding isozyme selective inhibitors of HDAC2. Further, I investigated the binding inclination and interaction mechanism of two plant-derived inhibitors apigenin and luteolin against Class I HDAC isozymes. Importantly, through hardcore Molecular Biology and Biochemistry approach we explored an entirely novel Plant based inhibitor against epigenetic therapeutic target from certain plant sources of Kashmir Valley. Different techniques like HEK-293T, HL-60 cell cultures, Pharmacological Intervention, Liquid chromatography-tandem mass spectrometry (LC-MS/MS), Western Blots, Molecular Docking/ MMGBSA, Real time PCR, Chromatin Immunoprecipitation (ChIP) followed by PCR, MTT assays,

DNA laddering assay and ChIP-sequencing were used to arrive at logical conclusion.

During this time that is from 2009 to the present day, I came across a lot of research, review papers, and books on HDACi. This made me quite aware of the current lacunae in the field. Thus, first I wrote a book on histone deacetylase inhibitors in the context of neurological disorders entitled **"Histone Deacetylase Inhibitors—Epidrugs for Neurological Disorders."** However, I was knowing that no single book is available that has thoroughly discussed histone deacetylase inhibitors in combined form with other conventional and molecular targeted agents, shortcomings of singlet therapy of HDACi and conventional therapies, toxicities associated with pan-HDAC inhibitors, and escalating need of isozyme selective inhibitors. These unavoidable facts resulted in the genesis of this book. Due to COVID-19 pandemic, the movement became restricted and I fruitfully spent this time in writing this precious book at home.



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Shabir Ahmad Ganai

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About the Author

Dr. Shabir Ahmad Ganai has formerly worked as Principal Investigator/Young Scientist in the University of Kashmir. He has over 9 years of working experience in the field of histone deacetylases and histone deacetylase inhibitors.

He has published more than 24 articles in highly reputed international journals, 3 international book chapters and has authored a book.

He is currently serving as a referee for various international journals like Scientific Reports, PLOS ONE, Medicinal Chemistry Research, Current Drug Targets, Current Topics in Medicinal Chemistry, and Journal of Agricultural and Food Chemistry (American Chemical Society Publications). He is a member of two international societies, including the Epigenetics Society.