

METHODS IN MOLECULAR BIOLOGY

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RNA Interference and Cancer Therapy

Methods and Protocols

Edited by

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Editor

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Preface

Even after decades of conventional research, the war against cancer is still continuing. At present, even though chemotherapy is the only key approach for cancer treatment, it fails to improve patient mortality and compliance due to poor bioavailability and severe dose-dependent debilitating effects on healthy tissues. Biological therapies have not been strong enough to replace conventional therapy in spite of being used to augment chemotherapies in the case of certain types of cancers. In the recent past, RNA interference (RNAi) has emerged as a safe and powerful therapeutic tool which has ushered in a new hope to combat cancer. It is an attractive approach for silencing specific genes responsible for disease onset and progression and thus offers enough scope to be employed as a potential therapeutic tool. Several techniques and procedures, both in vitro and in vivo, have been developed and adopted for assessing RNAi-based strategies in cancer treatment. However, the major challenges facing the success of RNAi-based drugs include the difficulty in their targeted delivery to the primary site of the tumor, stability, penetration, and its effective accumulation to therapeutic levels. Though scientists have achieved this by using viral-based vectors, the stigma attached with the viral pathogenicity that might be acquired due to mutations prevents its exploitation. Therefore, efforts are on to achieve effectiveness of viral-based delivery without compromising on the safety issues by employing non-viral agents. The main drawback of non-viral agents is the inherent toxicity associated with high transfection efficiency. Different types of nanoparticles developed offer solutions to ligand-targeted delivery of RNAi molecules to tumor cells, thus preventing off-target effects. The first clinical trial with siRNA molecules as therapeutics showed that these novel molecules can be safely dosed to humans. This also shows the need for development of new and improved formulations to make this revolutionary therapeutic a reality. Thus, RNAi is the future biological therapeutic molecule with immense potential and promise to move from bench to bedside, even though progress in the field is at a slow pace. Several interesting and useful procedures and methodologies have been developed by researchers working in this field which should act as a repertoire of techniques for adoption and exploitation by those pursuing RNAi as a fertile strategy to develop the bio-drugs of future. It is appropriate, timely, and relevant to collate such protocols to catalyze this process. Therefore, an attempt has been made here to collate the protocols available in this field, and I strongly feel that this compilation of 26 procedures with its relevant rationale, tips, and background information provides a backpack guide to researchers, both novice and professional, who intend to discover and innovate newer means of using RNAi technology to combat cancer, the scourge of human health.

Chapter 1 describes the protocol to identify synergistic combinations from siRNA libraries using robotic screen. Chapter 2 gives a simple and straightforward in vitro protocol to evaluate candidate gene knockdown for cancer therapy. Chapter 3 outlines a protocol for the development of siRNA-based drugs against apoptotic factor and its successful delivery to orthotopic models of prostate cancer. Chapter 4 provides the protocol for quantification of individual siRNA strand loading to Ago 2, a crucial parameter that determines efficient silencing. Chapter 5 explains efficient targeted silencing by non-viral vectors which contribute to

stable siRNA complex formation, specific intracellular uptake and release. Chapter 6 is about designing siRNA-encapsulating DNA nanosuitcases which conditionally release their cargo. Bio-drug in combination with chemo-drug as successful combinatorial therapy has been described in Chapters 7 and 8. Chapter 9 summarizes detailed protocol for preparation and targeted delivery of shRNAs using minicell (producing parent bacterial cells). Modified microRNAs have also been used to successfully target myeloid cells as narrated in Chapter 10. Different nanoparticle-based targeted deliveries including aptamers have been tried to combat shortcomings of viral-based deliveries, and the procedures adopted have been summarized in Chapters 11 through 17. As in the quote “the proof of the pudding is in the eating,” the success of RNA interference technology in combating various cancers undoubtedly has to come from the preclinical studies. Chapters 18–26 demonstrate precisely this and thus prove that an RNAi-mediated bio-drug is a futuristic molecule which holds promise for cancer therapy.

My sincere thanks to all the authors for sharing their detailed protocols with expertise and experiences described in each chapter. Special thanks to the series editor, John M. Walker, Professor Emeritus in the School of Life Sciences at the University of Hertfordshire, for his guidance during the editing process. It was an absolute pleasure to work with the editor David C. Casey at Springer.

Hyderabad, Telangana, India

Lekha Dinesh Kumar

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