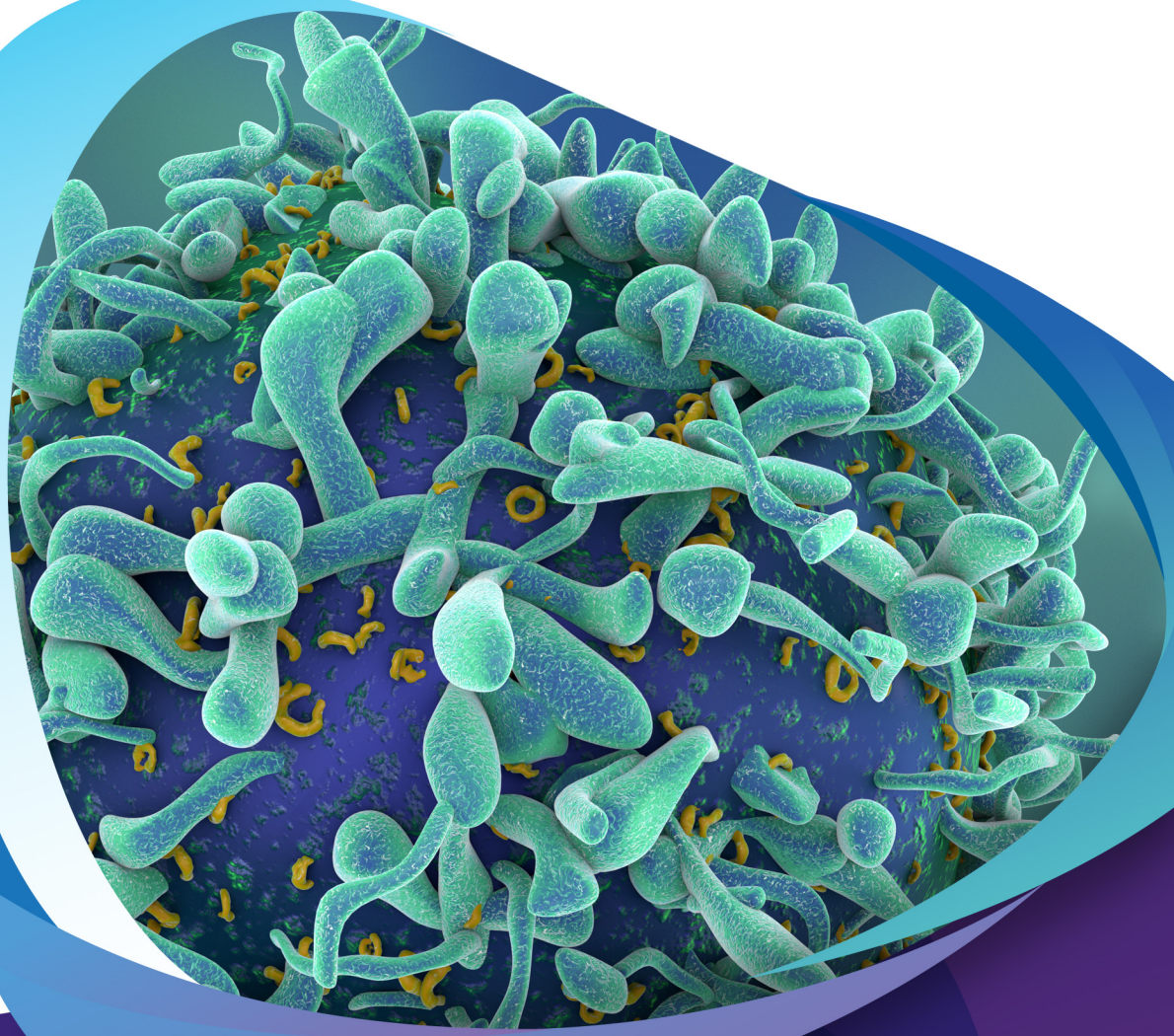
ADVANCES IN CANCER NANOTHERANOSTICS FOR EXPERIMENTAL AND PERSONALIZED MEDICINE



Editor:
Yusuf Tutar

Bentham Books

Advances in Cancer Nanotheranostics for Experimental and Personalized Medicine

Edited by

Prof. Dr. Yusuf TUTAR
University of Health Sciences,
Hamidiye Health Sciences Institute,
Division of Molecular Medicine
34668, Istanbul,
Turkey

and

Department of Basic Pharmaceutical Sciences,
University of Health Sciences,
Hamidiye Faculty of Pharmacy,
Division of Biochemistry
34668, Istanbul,
Turkey

Advances in Cancer Nanotheranostics for Experimental and Personalized Medicine

Editor: Yusuf Tutar

ISBN (Online): 978-981-14-5691-6

ISBN (Print): 978-981-14-5689-3

ISBN (Paper Back): 978-981-14-5690-9

need for a court order if at any point you breach any terms of this License Agreement. In no event will any delay or failure by Bentham Science Publishers in enforcing your compliance with this License Agreement constitute a waiver of any of its rights.

3. You acknowledge that you have read this License Agreement, and agree to be bound by its terms and conditions. To the extent that any other terms and conditions presented on any website of Bentham Science Publishers conflict with, or are inconsistent with, the terms and conditions set out in this License Agreement, you acknowledge that the terms and conditions set out in this License Agreement shall prevail.

Bentham Science Publishers Pte. Ltd.
80 Robinson Road #02-00
Singapore 068898
Singapore
Email: subscriptions@benthamscience.net

CONTENTS

FOREWORD

Oncologic drug development focused on single target-single drug strategy for several years. However, cancer cells are genius! They can bypass inhibitor perturbations by using alternative routes. Further, human genome project results indicated that four letter-alphabet is not as simple as central dogma; only few percent of the gene sequences are transcribed and translated. Rest of the genome function is involved in uncharacterized biochemical pathways and cell biology. And epigenetics and metabolites add more complexity to the understanding of molecular mechanisms in detail. This unknown mechanism makes cancer cell genius!!! But a great endeavor of eminent scientists and continuous research to elucidate pathways both in cellular and tissue levels make drug design more effective every day.

To control the effect of several cellular factors, the new trend in contemporary drug design is to employ drug cocktail that will synergistically act on these factors and proper oncologic drug targeting to eliminate off-targeting. For this purpose, nanocarriers have been designed to deliver drugs to tumor microenvironment not only to treat the tumor but prevent its metastasis.

Biomimicking is an old fashioned yet excellent method in disease treatment. Macrophages target cancer cells and using this biomimick bullet macromolecule with oncologic drug cargo serves as a fine treatment strategy. The biomimick bullet cargo can be a wide range of molecules from small to large molecules.

Targeting with nanotheranostic carriers provides specific delivery to cancer microenvironment. However, cancer cells so called “the other strategies” yet to be elucidated. For example, transformation of a healthy cell to cancer cell may enhance inner and surface signaling molecules and may introduce new set of metabolites. Further, some of the non-coding gene’s expression increases during this transformation. Altogether, cellular and tissue level characterization of oncologic pathways may help our understanding of tumor biology. Currently, cancer nanotheranostics tries to find a short cut for experimental and personalized medicine in cancer treatment. This book covers recent advances in this field.

Prof Lütfi TUTAR
Ahi Evran University
Turkey

PREFACE

Personalized medicine with novel therapeutic approaches provides direct targeting of macromolecules with contemporary drug delivery systems for treatment of severe diseases, including cancer. Nanotheranostic design offers increased bioavailability of the drugs through controlled release and distribution. Nanotheranostics also integrates diagnostic test with treatment of the disease. Recent advances in cancer studies revealed new genetic elements and factors that affect theranostic drug targeting approaches. Also, several tumors are challenging, and new treatment modalities are required. Molecular level mechanisms provide valuable information for therapy and innovative design for treatment. Several creative approaches have been proposed for theranostic therapy. For this reason, an updated approach over *in vivo* and translational properties of nanotheranostics with special emphasis on cancer will widen the scope of the readers/researchers with this book.

Chapter 1 despite significant advances in cancer therapy, many tumors are still challenging, and novel strategies are essential for treatment. Nanotheranostics use nanotechnology for diagnosis and therapy of cancer. Recent advancement in nanotechnology has provided novel types of nanomaterials composed of either organic- or polymer-based nanoparticles. Small alterations and modifications transform this carrier system with unique properties and optimize drug delivery and release. This chapter provides overview in cancer nanotheranostics field.

Chapter 2 overviews tumor microenvironment as prelude. This site regulates tumor progression and metastasis. Non-cellular components in this environment such as cytokines, chemokines, growth factors, inflammatory and matrix remodeling enzymes shape the progression of the disease by mediating the communication taking place between the tumor itself and its surrounding. This may prevent the benefits of therapeutical strategies. The chapter focuses on understanding the function and mechanism of these non-cellular components in the environment to elucidate obstacles in the treatment of cancer.

Chapter 3 covers immune system employment in fighting cancer cells to prevent tumor development. Immunotherapies are innovative cancer treatment. Nanomedical formulations modulate macrophages which can influence the tumor microenvironment, since macrophages target tumor environment. Macrophage may be used as trojan horse and its cargo may mediate gene and/or protein expression in the treatment regime. This section discusses improvements in cancer immunotherapies through this biological strategy.

Chapter 4 Gene and genome modification tools allow gene therapy through alteration of malignant genes and editing mutations for correction of errors. These innovative technologies deliver therapeutic nucleic acids to cells and tissues. Therefore, the success of gene therapy formulation is proportional to efficient delivery of the carrier and its nucleic acid cargo to a specific target and proper cellular uptake. The platforms have been developed for higher loading capacity, and low immunogenicity and toxicity. In chapter 4, the authors provide a review on different gene delivery vectors and platforms at the nanoscale.

Chapter 5 Oncology research applications may not yet fully suppress cancer-based mortalities and morbidities. Conventional therapeutic approaches have limitations as most research depends on coding genes. Human genome sequencing revealed that only 2-3 percent of the genome codes for genes and proteins however the rest is unknown. Further, heterogeneity among malignant tumors lead obstacles. Therefore, “precision medicine” in oncology and its extrapolation to “personalized treatment” for each cancer patient is essential. The chapter

covers non-coding RNAs as biopharmaceutical tools in oncology. The new trend in drug design is covered in this section.

Chapter 6 Nanoparticles are convenient carrier systems based on their plasmonic and magnetic properties, active surface areas and various physicochemical properties. Development of therapeutic nanoparticles provides imaging modalities such as magnetic resonance imaging, radionuclide-based imaging; positron emission tomography and single-photon emission computed tomography and X-ray-computed tomography. Methodology and applications of the techniques are explained thoroughly in this chapter.

Chapter 7 covers practical clinical applications in chemotherapy and nuclear medicine. The simultaneous yield of imaging in radiologic and nuclear medicine applications and therapeutic agents offers diagnosis and treatment effectiveness in real-time.

This book covers recent advancements both in applied and in clinical research. Since targeting small organic molecules are common, the book mainly focused on DNA, protein and immunotherapy on cancer. Different applications for cancer treatment are in progress but basic strategies are similar. We hope this book will help not only early career scientists but also will help experienced researchers to widen the scope of their projects.

Yusuf TUTAR
University of Health Sciences
Istanbul
Turkey

List of Contributors

Ezgi Nurdan Yenilmez Tunoglu	University of Health Sciences, Hamidiye Health Sciences Institute, Division of Molecular Medicine, 34668, Istanbul, Turkey
Berçem Yeman	University of Health Sciences, Hamidiye Health Sciences Institute, Division of Molecular Medicine, 34668, Istanbul, Turkey
Merve Biçen	University of Health Sciences, Hamidiye Health Sciences Institute, Division of Molecular Medicine, 34668, Istanbul, Turkey
Servet Tunoglu	Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, 34093, Istanbul, Turkey
Yousef Rasmı	Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, 571478334, Urmia, Iran
Yusuf Tutar	University of Health Sciences, Hamidiye Health Sciences Institute, Division of Molecular Medicine, 34668, Istanbul, Turkey University of Health Sciences, Hamidiye Faculty of Pharmacy, Department of Basic Pharmaceutical Sciences, Division of Biochemistry, 34668, Istanbul, Turkey
Raghda Ashraf Soliman	Pharmaceutical Biology Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, 11835, Cairo, Egypt
Rana Ahmed Youness	Pharmaceutical Biology Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, 11835, Cairo, Egypt
Mohamed Zakaria Gad	Biochemistry Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, 11835, Cairo, Egypt
Asmaa Mostafa	Department of Internal Medicine III, University Hospital RWTH Aachen, Pauwelsstraße 30, 52074, Aachen, Germany Department of Microbial Biotechnology, Division of Genetic Engineering and Biotechnology, National Research Center, 33 El-Bohouth St., El-Dokki, 12622, Giza, Egypt
Matthias Bartneck	Department of Internal Medicine III, University Hospital RWTH Aachen, Pauwelsstraße 30, 52074, Aachen, Germany
Beatriz B. Oliveira	UCIBIO, Life Sciences Department, Faculdade de Ciências e Tecnologia, Campus de Caparica, 2829-516 Caparica, Portugal
Alexandra R. Fernandes	UCIBIO, Life Sciences Department, Faculdade de Ciências e Tecnologia, Campus de Caparica, 2829-516 Caparica, Portugal
Pedro V. Baptista	UCIBIO, Life Sciences Department, Faculdade de Ciências e Tecnologia, Campus de Caparica, 2829-516 Caparica, Portugal
Seda Keleştemur	University of Health Sciences, Institution of Health Sciences, Department of Biotechnology, Tıbbiye Cad., 34668, Istanbul, Turkey
Gamze Kuku	Yeditepe University, Faculty of Engineering, Department of Genetics and Bioengineering, Kayısdagı Cad., 34755, Istanbul, Turkey
Turkan Ikizceli	Department of Radiology, University of Health Sciences Turkey, Haseki Training and Research Hospital, Istanbul, Turkey

S. Karacavus Department of Nuclear Medicine, University of Health Sciences Turkey, Kayseri City Training and Research Hospital, Kayseri, Turkey

CHAPTER 1

Cancer Nanotheranostics

Ezgi Nurdan Yenilmez Tunoglu[1], Berçem Yeman[1], Merve Biçen[1], Servet Tunoglu[2], Yousef Rasmi[3] and **Yusuf Tutar[1,4,*]**

[1] *University of Health Sciences, Hamidiye Health Sciences Institute, Division of Molecular Medicine, 34668, Istanbul, Turkey*

[2] *Department of Molecular Medicine, Aziz Sancar Institute of Experimental Medicine, Istanbul University, 34093, Istanbul, Turkey*

[3] *Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, 571478334, Urmia, Iran*

[4] *Department of Basic Pharmaceutical Sciences, University of Health Sciences, Hamidiye Faculty of Pharmacy, Division of Biochemistry, 34668, Istanbul, Turkey*

Abstract: Molecular profiling of diseases identifies specific cancer-causing genes and associated networks. Administered drug displays different therapeutic efficiency depending on individual cancer subtype and therapeutic responses. Personalized medicine helps designing treatment methods for individual patients with distinct diseases. For complete understanding of patient's pathophysiology, different omics data types are integrated. These data can be derived from whole-exome sequencing, metabolomics, pharmacogenomics, and proteomics. Pharmacogenomics deals with the interaction of drug and patient's genetic make-up and metabolomics reveals custom regulation of biochemical pathways in patients. Transcriptomics and proteomics analyze organism tissue or cell type in cancer and play even more relevant role in personalized medicine. Since associated genetic anomalities and metabolic profiles influence therapy response, a continuous evolution of cancer nanotheranostics helps preventing and treating the disease more precisely.

Keywords: Cancer, Metabolomics, Nanotheranostics, Personalized medicine.

INTRODUCTION

There is a six million nucleotide difference between two individual's genome and even twins have 35 intergenerational mutational nucleotide differences [1]. Epigenetic alterations as well as gene duplication or deletion like unequal crossing over alter genomic content during the course of individual life cycle.

* **Corresponding author Yusuf Tutar:** University of Health Sciences, Hamidiye Health Sciences Institute, Division of Molecular Medicine, 34668, Istanbul, Turkey and Department of Basic Pharmaceutical Sciences, University of Health Sciences, Hamidiye Faculty of Pharmacy, Division of Biochemistry, 34668, Istanbul, Turkey;
E-mail: yusuf.tutar@sbu.edu.tr

Therefore, nanotheranostic approaches require delivery of individually adapted medicine based on genetic profiles of cancer patients. High-throughput technologies in oncology provide genomic analysis to be used for guidance of individualized medical treatment.

Initial studies of individualized treatment started with the Human Genome Project (HGP). HGP has helped to make use of the relationship between drug and target and improve its efficacy and safety. In this concept, patients are individually treated taking their unique genomic profiles into consideration. Even though the genomic profiles of different cells of a patient are the same, their expression profiles are clearly different. This difference is taken into account in personalized medicine along with the patient's disease history. On the other hand, traditional treatment methods have overlooked the genetic variability among patients and focused on a reactive approach based on population-based conclusions. During the physical examination; symptoms described by patients, medications taken, and biopsy outcomes are taken into account to decide on the traditional method to be implemented [2].

A new concept, P4 medicine, was first introduced in 2011 by Hood *et al*. as a systems approach including predictive, preventive, personalized, and participatory features of medicine. This approach puts emphasis on the patient instead of the disease itself, making it a proactive discipline rather than reactive [3]. P4 medicine does not focus just on genomic data but also involves data from DNA (together with epigenetic changes), RNA, protein, metabolite, cell, and tissue level. Using the data gathered, it is possible to personalize any form of treatment for any form of disease. It is important to have as much knowledge as possible of each patient's and tumor's genetic backgrounds to increase the efficiency of targeted treatment. When the two pieces of information are evaluated with regard to one another, it is also possible to determine the risk groups for specific disease types. Overall, personalized medicine aims to design the right treatment for the right patient at the right time and the right dose.

ONCOLOGY IN BRIEF

Our body is a living and growing system that contains billions of cells that perform many functions such as metabolism, transport, secretion, reproduction and mobility. Growth and development occur as a result of the growth of newly formed cells and their transformation into different types of tissues. The branch of oncology is interested in cancer and the biochemistry of cancer cells is different. There are three different types of cells in our body: static cells (differentiated cells), growing cells (undifferentiated cells) and regenerating cells (stem cells). In contrast to these cell types, cancer cells do not have a growth-inhibiting control

mechanism as in normal cells. Therefore, it is possible to compare cancer cells to uncontrolled stem cells. Tumors can be malignant or benign tumors. Cancer occurs due to many factors. In addition to genetic factors, many environmental factors such as UV light, X-rays, chemicals and tobacco products can cause cancer. In order to define cancer, it is necessary to understand cancer genetics. There are three types of genes in cancer genetics: Oncogenes, tumor suppressors and DNA repair genes. The normal form of an oncogene is defined as a proto-oncogene. Proto-oncogenes are converted to oncogenes by mutation. Tumor suppressors produce proteins that avoid cell division and cause cell death. Genes that prevent cancer-causing mutations are DNA repair genes. Occasionally, a virus-induced mechanism inserts nucleotides into or near a proto-oncogene and transform it to an oncogene. This results in uncontrolled cell growth. A single oncogene is usually not adequate to cause cancer. Cancer-related genes [4] serve as a biomarker in the definition of cancer (Table **1**).

Table 1. Some genes associated with cancer.

Name	Function	Examples of Cancer/Diseases	Type of Cancer Gene
APC	regulates transcription of target genes	Familial Adenomatous Polyposis	tumor suppressor
BCL2	involved in apoptosis; stimulates angiogenesis	Leukemia; Lymphoma	oncogene
BLM	DNA repair	Bloom Syndrome	DNA repair
BRCA1	may be involved in cell cycle control	Breast, Ovarian, Prostatic, & Colonic Neoplasms	tumor suppressor
BRCA2	DNA repair	Breast & Pancreatic Neoplasms; Leukemia	tumor suppressor
HER2	tyrosine kinase; growth factor receptor	Breast, Ovarian Neoplasms	oncogene
MYC	involved in protein-protein interactions with various cellular factors	Burkitt's Lymphoma	oncogene
p16	cyclin-dependent kinase inhibitor	Leukemia; Melanoma; Multiple Myeloma; Pancreatic Neoplasms	tumor suppressor
p21	cyclin-dependent kinase inhibitor		tumor suppressor
p53	apoptosis; transcription factor	Colorectal Neoplasms; Li-Fraumeni Syndrome	tumor suppressor

CHAPTER 2

Tumor Microenvironment: A Critical Determinant in Regulating Tumor Progression and Metastasis

Raghda Ashraf Soliman[1], Rana Ahmed Youness[1,*] and **Mohamed Zakaria Gad[2,*]**

[1] *Pharmaceutical Biology Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, 11835, Cairo, Egypt*

[2] *Biochemistry Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, 11835, Cairo, Egypt*

Abstract: The fate of cancer cells is predicted not only by its intrinsic oncogenic engines, but also by its surrounding milieu. Beyond the tumor margin at the tumor microenvironment (TME), there is an orchestra of immune cells and soluble mediators known as the cellular and non-cellular components of TME that shape the tumor architecture. Several reports have focused on immune cells influencing the cellular components of the TME, therefore the main focus of our chapter will be the non-cellular components of TME. The non-cellular components of TME include cytokines, chemokines, growth factors, inflammatory and extra-cellular matrix remodeling enzymes that are released by the tumor cells or associated immune cells in the TME. These soluble mediators outline the progression of the disease by mediating the communication taking place between the tumor cell itself and its surrounding. Considering that TME is a critical determinant in unraveling the complexity of cancer cells, thus, zooming in at the TME would definitely help us pave the road for new combinatory immuno-oncological interventions incorporating the TME in their mechanism of action and thus lowering the chances of relapse rates among cancer patients.

Keywords: Angiogenesis, Angiogenic switch, Cancer Associated Fibroblasts (CAF), Cytokines, Chemokines, Extracellular Matrix (ECM), Growth factors (GFs), Hypoxia, Hypoxia inducing Factor-1 (HIF-1), Inflammation, Immune surveillance, Remodeling enzymes, Tumor microenvironment (TME), Tumor Infiltrating Lymphocytes (TILs), T cell exhaustion, Tumor associated Macrophages (TAMs).

[*] **Corresponding authors Dr. Rana Ahmed Youness:** Pharmaceutical Biology Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, 11835, Cairo, Egypt; E-mails: rana.ahmed-youness@guc.edu.eg; rana.youness21@gmail.com, **Prof. Mohamed Zakaria Gad:** Biochemistry Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, 11835, Cairo, Egypt; Tel: 002-01272222695; Fax: +202-2-2759 0711; E-mail: Mohamed.gad@guc.edu.eg

Yusuf Tutar (Ed.)

THE TUMOR MICROENVIRONMENT

Cancer is not just abnormal cells that continue to grow and metastasize, it is a complex tissue made up of tumor cells and their surrounding stroma [1]. These tumor cells can communicate with the cellular and non-cellular components in their surroundings. This is referred to as the tumor microenvironment (TME) [2]. TME can modulate the tumor's aggressiveness, rate of growth, immune recognition and metastatic properties [3].

The cellular components of the TME have been extensively studied. They mainly include T-lymphocytes [4, 5], $CD4^+$ T cells [6, 7], $CD8^+$ T cells [8, 9], regulatory T cells (Tregs) [10], B-lymphocytes, natural Killer (NK) cells [11], mesenchymal stem cells (MSCs) [12], tumor-associated-macrophages (TAMs) [13, 14], myeloid derived suppressor cells (MDSCs) [15 - 17], tumor-associated neutrophils (TANs) [18, 19], and terminally differentiated myeloid dendritic cells (DCs) [20], pericytes [21], adipocytes [22, 23], and cancer associated fibroblastic cells (CAFs) [24, 25] as shown in Fig. (**1**). The non-cellular TME components include cytokines, chemokines, growth factors, inflammatory and extracellular matrix remodeling enzymes; all of which play role in the communication taking place between the tumor itself and its surroundings [26]. In this chapter, we will be mainly discussing the cellular and non-cellular components of TME and their integrated interplay to dictate the fate of tumor cells. Nonetheless, we will shed light on the potential role of TME in the immune-therapeutic equation and its consideration in the new combinatory therapeutic intervention evading the immuno-oncology market.

CELLULAR COMPONENTS OF THE TME

The immune setting within the TME significantly determines cancer fate. A strong lymphocytic infiltration has been paradoxically reported to be associated with good or poor clinical outcomes in different human tumors depending on the type of immune cells infiltrating the TME [27 - 29].

Tumor Infiltrating Lymphocytes

The term tumor-infiltrating lymphocytes (TILs) was first made up by Wallace Clark in 1969 and later defined operationally as a lymphocyte that has migrated from bloodstream towards tumor cells. More recently, the term TILs has been used to describe a variety of tumor-infiltrating cells including T cells, B cells, macrophages, DCs, NK cells, and MDSCs [30].

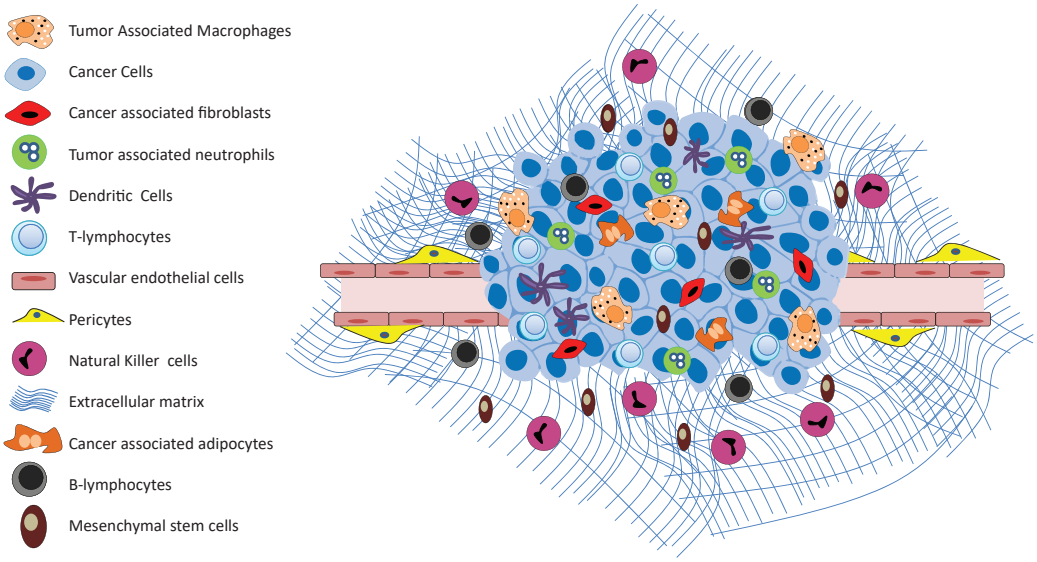


Fig. (1). Cellular components of the tumor microenvironment: Tumor Associated Macrophages (TAMs), Cancer Associated Fibroblasts (CAFs), Tumor Associated Neutrophils (TANs), Dentritic Cells (DCs), T-lymphocytes, Vascular endothelial cells, pericytes, Natural Killer cels (NKs), Cancer associated adipocytes, B-lymphocytes, Mesenchymal Stem Cells (MSCs) and Extracellular matrix of the tumor.

T-lymphocytes

T cells are divided into two types according to their clusters of differentiation (CD); $CD4^+$ T (helper T cells, Th) and $CD8^+$ T (cytotoxic T cells, Tc) cells. $CD4^+$ cells further compromise subtypes besides the classical T-helper 1 (Th1) and T-helper 2 (Th2) cells [31, 32]. These include T-helper 17 (Th17), follicular helper T cell (Tfh), induced T-regulatory cells (iTreg), and the regulatory type 1 cells (Tr1) as well as the potentially distinct T-helper 9 (Th9) [33 - 35]. This differentiation is due to the complex cytokine signaling network, transcription factors, and epigenetic alterations [36]. Tumors have the ability to suppress T cells once they migrate into the tumor margin or infiltrate a tumor tissue where they encounter the TME [37]. Three signals are required for T cell activation. At the tumor-immune synapse, first, the MHC-peptide complex is recognized by T cell receptor. Then, the pairing of co-stimulatory or co-inhibitory molecules occurs, and finally, proper soluble mediators are produced. Of these signals, the pairing step to co-inhibitory/stimulatory molecules is crucial in mounting the cytokine profile responsible for the differentiation of lymphocytes into either activator or inhibitor phenotypes. In normal scenarios, co-inhibitory molecules such as PD-1 and CTLA-4 inhibit inflammation and prevent the unfortunate increase of immune system responses and tissue damage. However, in cancer, an excessive rise of co-inhibitory signals takes place wearying immune responses

CHAPTER 3

Nanotechnology in Cancer Theranostics

Asmaa Mostafa[1,2] and **Matthias Bartneck**[1,*]

[1] *Department of Internal Medicine III, University Hospital RWTH Aachen, Pauwelsstraße 30, 52074, Aachen, Germany*

[2] *Department of Microbial Biotechnology, Division of Genetic Engineering and Biotechnology, National Research Center, 33 El-Bohouth St., El-Dokki, 12622, Giza, Egypt*

Abstract: Our immune system protects our body from a large number of threats. External threats include pathogens from various sources. Internally, the cells of our immune system continuously fight cancer cells and thereby prevent tumor development. Immunotherapies which employ monoclonal antibodies have significantly enriched our vision of cancer treatment. Unleashing the checkpoint blockade of tumors mobilizes the cytotoxic T cells to eliminate cancer cells, and therefore, amplifies the anti-tumor response of the immune system. The lymphoid immune cells, particularly cytotoxic CD8 T cells, are the current focus of novel interventions such as chimeric antigen receptor (CAR) engineered T cells. Nanomedicines are predestined to target macrophages due to their high phagocytic activity and their large numbers in different types of tumors. Specifically, nanomedical formulations might additionally explore the potential of modulating macrophages as key effector cell which can influence the tumor microenvironment. The therapeutic cargo to be delivered to cells or tissues can benefit from the "Omics" sciences and use knowledge to specifically modulate gene expression and protein generation using small non-coding RNA. Strategies to localize drug delivery have the potential to enrich nanomedicines for their potential ability to be concentrated in certain parts of the body. Such applications can rely, for instance, on magnetic fields or infrared light sensitive systems, in order to increase target specificity. Here, we put an emphasis on the applicability of the strategies to improve target specific accumulation of theranostics and discuss potential improvements of cancer immunotherapies.

Keywords: Cancer immunotherapy, Drug delivery, Imaging, Nanomedicine, Theranostic.

[*] **Corresponding author Matthias Bartneck:** Department of Medicine III University Hospital Aachen 52074 Aachen, Germany; Tel: -49-241-80662; Fax: -49-241-82455 Email: mbartneck@ukaachen.de

Yusuf Tutar (Ed.)

NANOMEDICINES AND THERANOSTICS

1. Putative Anti-cancer Nanomedicines and their Biodistribtion

Nanomedicines, nanotechnologically generated drugs, cover a broad range of sizes of a few up to several hundred nanometers [1]. Depending on the nature of the material, they can be classified into two major groups, organic or inorganic. In many cases, the organic particulates are clinically usable and the inorganic formulations are mostly used in research. Gold nanoparticles (AuNP) probably are the most intensively studied type of inorganic nanoparticle. AuNP can easily be altered in terms of size, shape, and functionalization such as nanorods [2], nanocages [3], or nanostars [4]. The metallic nature allows for optical and magnetic properties to be traced in whole body-based and cellular imaging [5, 6]. However, a drawback of inorganic nanoparticles is given by their temporal accumulation in the body, owed to their missing degradability. Earlier own studies have demonstrated that gold nanorods reside in the liver to a similar extent after seven days compared to the level after one day [5]. Importantly, not all organic nanoparticles, for instance fullerenes or carbon nanotubes [7], are biodegradable.

A huge number of organic nanoparticles have been generated. Importantly, liposomes and the particles based on polymers such as N-(2-Hydroxypropyl) methacrylamide (HPMA) belong to the most successful formulations [8]. Most organic nanoparticles exhibit the major advantage of being biodegradable by means of their composition. For instance, liposomes can be integrated into the cell membrane which also contain phospholipids or cholesterol [9]. Most nanoparticles, in particular AuNP [5], but also iron oxide-based formulations, are non-toxic at clinically usable concentrations [10]. Importantly, very small AuNP of size 1.4 nm are toxic to cells [11]. Nevertheless, the dose determines the toxicity and at high doses, many nanotherapeutics can be toxic as demonstrated for titanium dioxide nanoparticles [12]. Certain materials can be toxic, *i.e*. silica-based nanoparticles exhibit immunotoxicity by activating macrophages [13, 14]. To reduce the adherence of serum proteins to therapeutics, PEGylation, the decoration with a PEG layer, is carried out to reduce the unspecific internalization by phagocytes based on a neutral charge which repels many types of proteins [15]. Specific functionalizations, such as peptides [16], can affect an immune response, such as that of macrophages and dendritic cells [5, 16, 17].

Liposomal formulations are the most successful ones, based on their market size. Novel formulations continuously enter the market and recently, Vyxeos gained approval for the treatment of acute myeloid leukemia (AML) in August 2017. Vyxeos delivers both cytarabine and daunorubicin at a molar ratio of 5:1 [18]. Vyxeos showed an improvement in the efficacy in two phase 2 clinical trials,

compared with a standard cytarabine and daunorubicin regimen [19, 20].

Promising novel clinical trials are those on drugs with a temperature-inducible drug release, specifically Thermodox (Celsion Corp.) [21]. This liposomal doxorubicin is prepared with thermally sensitive lipids that degrade on exposure to high heat and disrupt the lipid bilayer, evoking drug release [21, 22]. The combination of nanodrug with radiofrequency thermal ablation allows the drug to be released in a site-specific manner at the tumor [21, 22]. Many clinical trials in phase 3 on combined Thermodox and radiofrequency ablation technique have been completed or are still in progress [22].

Polymers can be natural, synthetic, or pseudosynthetic [23]. Polyethylene glycol (PEG) is the most frequently used polymer [21]. Polymeric nanomedicines form an important pillar in nanomedicine since many studies have reported their high efficacy, safety, and prolonged drug release [21, 24]. As a result, polymeric NPs seem to be promising nano-carriers for various medications [24]. Polymeric NPs can be formulated by adding agents to their surface that can be utilized in diagnostic imaging [24]. Biodegradable polymers have attracted the attention of many researchers in recent years since they can be completely metabolized and thus removed from the body [24]. PLGA (polylactic-glycolic acid) is an interesting biodegradable polymer based on its relative proportions of polylactic acid (PLA) and polyglycolic acid. The ratio between both constituents can be used to modulate the biodegradability behavior of PLGA [24].

Micelles are self-assembling polymeric NPs with a hydrophobic internal core used to encapsulate drugs that have a low degree of solubility in aqueous solution, while the external surface of a micelle has enough polarity to allow dissolution in aqueous media [21]. The drugs delivered by micelles include many hydrophobic drugs, including anti-cancer medicines [25 - 28]. Polymeric micelles can deliver their cargo using both passive and active targeting capabilities. Active targeting of specific receptors can enhance the selectivity of drug delivery and thus potentially decreases the side effects [29]. For tumor targeting, micellar NPs utilize the so-called enhanced permeability and retention (EPR) effect [30, 31] for passive accumulation, while they make use of certain ligands, such as antibodies or folic acid [32] for deliberate active targeting. Stealth micelles sizing 10–100 nm are large enough to avoid excretion through the kidneys, and at the same time they are sufficiently small to overcome filtration in the spleen. In sum, this leads to a prolonged circulation time of these particles [33]. Therefore, polymeric micelles enable a directed delivery of higher drug doses to target sites while reducing systemic toxicity [32].

Micelle-based Estrasorb is FDA approved for vasomotor-associated symptoms

CHAPTER 4

Nanotheranostics in Gene Therapy

Beatriz B. Oliveira, Alexandra R. Fernandes and **Pedro V. Baptista***

UCIBIO, Life Sciences Department, Faculdade de Ciências e Tecnologia, Campus de Caparica, 2829-516 Caparica, Portugal

Abstract: The continuous advances in molecular genetics have prompt for a wealth of tools capable to modulate genome and the corresponding gene expression. These innovative technologies have broadened the range of possibilities for gene therapy, either to decrease expression of malignant genes and mutations or edition of genomes for correction of errors. These strategies rely on the delivery of therapeutic nucleic acids to cells and tissues that must overcome several biological barriers. Indeed, a key element for the success of any gene therapy formulation is the carrier agent capable to deliver the therapeutic nucleic acid moieties to a specific target and promote efficient cellular uptake, while preventing deleterious off-target effects and degradation by endogenous nucleases. The initial vectorization strategies proved to be rather immunogenic, limited in the amount of genetic material that can be packed and raised severe toxicity concerns. Nowadays, a new generation of nanotechnology-based gene delivery systems are making an impact on the way we use therapeutic nucleic acids. These nanovectorization platforms have been developed so as to show low immunogenicity, low toxicity, ease of assembly and scale-up with higher loading capacity. Some of these nanoscale systems have also allowed for controlled release system and for the simultaneous capability of monitorization of effect – nanotheranostics. Herein, we provide a review on the variety of gene delivery vectors and platforms at the nanoscale.

Keywords: Cancer, Gene therapy, Nanomedicine, Nanotheranostics, RNAi.

GENE DELIVERY FOR CANCER THERAPY

In 2016, cancer accounted for more than 16% of the all deaths worldwide [1], with surgery, radiotherapy, chemotherapy, immune or hormone therapy being the standard procedures to manage tumors caused by gene mutations, deficiency or overexpression. Despite their valuable application, 95% of all new therapeutics show poor pharmacokinetics and/or biopharmaceutical properties and side effects

* **Corresponding author Pedro V. Baptista:** UCIBIO, Department Life Sciences, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516 Caparica, Portugal; Tel: +351 21 2948530; E-mail: pmvb@fct.unl.pt

Yusuf Tutar (Ed.)

that may limit their clinical application [2]. Hence, the ultimate challenge is to develop effective, nontoxic, non-immunogenic, noncarcinogenic vectors to deliver nucleic acids and/or drugs into cells, to achieve selective delivery of therapeutics to target areas in the body, minimizing their side effects and increasing their efficiency [3, 4]. Hence, the approval of novel gene/drug delivery platforms for cancer management is now a critical need [5].

Broadly, gene therapy consists in the manipulation of cells genetic information at the nucleic acid level, for the correction or modification of defective and/or missing gene sequences, so as to cure inherited and/or acquired diseases [3, 6]. In the last decades, with the discovery of novel small RNA molecules and their important role in the regulation of cell function, RNA has become both the target and the effector of a range of novel therapeutics [7]. RNA therapeutics refers to the use of oligonucleotides (DNA or RNA) to target RNA molecules for therapeutic needs. Currently, there are two major approaches in RNA therapeutics that have already been tested in the clinics: double-stranded RNA-mediated interference (RNAi) and antisense oligonucleotides (ASO) [8]. ASOs act *via* bind to their nucleic acid target by Watson-Crick base paring, inhibiting or altering the gene expression through steric hindrance, initiation of target degradation, splicing alterations or other events. RNAi operates *via* sequence specific and post-transcriptional modulation, preventing translation into proteins, through the activation of ribonucleases and other enzyme complexes that co-ordinately degrade the targeted RNA molecules [9]. RNAi therapeutics can be achieved though the design of specific RNAi regulators, such as microRNAs (miRNAs) mimics and small interfering RNAs (siRNAs). Antagomirs are novel synthetic single-stranded miRNA analogues with 21-23 nucleotides of range, that have been recently developed specifically for miRNA silencing. The use of this native mechanism for therapeutic and diagnostic purposes can be achieved by exogenous delivery of synthetic RNAi molecules. However, due to their negative charge and relatively large size, RNAi molecules are less likely to readily cross the biological barriers. Therefore, carrier systems and chemical modifications are needed, to protect the RNAi molecules, allowing an effective systemic delivery to the site of action. Importantly, carrier systems provide a simplified manner of cell penetration maintaining the functionality of RNAi molecules [10, 11].

More recently, a new class of nucleic acid-based therapeutics have been proposed for application in a wide range of diseases - precision genome editing using the clustered regularly interspaced short palindromic repeats (CRISPR)-CRISPR associated 9 (Cas9) technology [12]. Indeed, with Cas9, a RNA-guided endonuclease, it is possible to engineer DNA double strand breaks (DSB) at specific *loci*, thus editing the genome at precise locations. Several applications of CRISPR technology for gene inactivation and genome editing have been reported

in humans [13]. This CRISPR-Cas9 strategy was preceded by other editing schemes that rely on altering endogenous molecular machinery to edit target regions of genomic DNA, such as zinc fingers nucleases (ZFN, that fuse the capability of zinc fingers domains to recognized DNA target sequences and nuclease activity), transcription activator-like effector nucleases (TALEN, that fuse a TAL effector DNA-binding domain to a DNA nuclease domain), among others [14 - 16]. Still, these pioneering concepts have had a small impact beyond preliminary studies as proof-of-concept and, as such, not yet translated to the clinical setting.

Having all these new technologies available is of cumbersome relevance, but a key element is crucial for the success of any gene therapy formulation: the carrier agent. Primary challenges are the delivery of the therapeutic nucleic acid moieties to a specific target and the efficient cellular uptake [17]. First generation of carriers were viral vectors, capable to mediate gene transfer with high efficiency and provide for long-term gene expression. However, they are rather immunogenic, show limitations in the amount of genetic material that can be packed and have raised severe toxicity concerns [18]. These drawbacks have prompted for development of non-viral alternatives for gene delivery showing low immunogenicity, low toxicity, ease of assembly and scale-up, and higher loading capacity [19]. The critical parameters of the carrier include size, shape, ligand functionalization and surface charge, that dictate the level of protection of the therapeutic nucleic acid against degradation and the pathway of cell uptake [18]. Moreover, a successful approach should include a controlled release system. This paved the way for the development and optimization of nanotechnology-based delivery of gene therapy. Herein, we shall provide a review on the diversity of gene delivery vectors and platforms at nanoscale and their applications.

NANOTECHNOLOGY IN GENE DELIVERY

Nanotechnology (and/or nanoscale) systems show unique properties that have been explores towards the development of conceptual therapeutic and diagnostic platforms, including *in vivo* imaging of drug delivery [20]. Some of these concepts have been further developed and several clinical trials demonstrate the powerful impact of nanotechnology-based systems in molecular therapeutics. Perhaps, the strongest impact has been felt in the field of cancer therapeutics, where nanomedicine has been showing great promise for controlled and targeted delivery of drugs and molecular actuators for gene therapy. Understanding the molecular causes of cancer had a major contribute on the design of targeted gene delivery [21].

CHAPTER 5

Short Non-coding RNAs: Promising Biopharmaceutical Weapons in Breast Carcinogenesis

Rana Ahmed Youness[1,*] and **Mohamed Zakaria Gad**[2,*]

[1] *Pharmaceutical Biology Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, New Cairo City, Main Entrance Al Tagamoa Al Khames, 11835, Cairo, Egypt*

[2] *Biochemistry Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, New Cairo City, Main Entrance Al Tagamoa Al Khames, 11835, Cairo, Egypt*

Abstract: Despite being previously annotated as 'junk' transcriptional products, the non-coding RNA molecules (ncRNAs) have proven their indisputable role in carcinogenesis. ncRNAs are believed to act as potent oncogenic mediators or tumor suppressors in different contexts in oncology. Functionally, ncRNAs are able to modulate various processes in the cell such as chromatin re-modeling, transcription, post-transcriptional modifications and especially signal transduction. The most abundant and well-studied ncRNA molecules are the microRNAs (miRNAs/miRs). Different oncogenic signaling cascades have recently been in relation with miRNAs in a bi-directional crosstalk. Thus, this chapter offers a wider perspective towards complex networks of interactions coordinated by miRNAs specifically in Breast Cancer (BC). Nonetheless, this chapter also sheds the light onto clinical status of the miRNAs as a potential therapeutic intervention in several contexts.

Keywords: Breast Cancer, MicroRNAs, miR-34a, miR-21, MRX34, RG-012.

IN ONCOLOGY: IT IS NOT "ONE SIZE FITS ALL"

In-spite of the enormous efforts directed towards oncology research, its incidence rate and the number of cancer-related mortalities are still steeply rising [1], thus ringing a bell for a lot of limitations in the conventional therapeutic approaches and treatment protocols currently available for cancer patients. After decades of research and enormous observations, a new hypothesis is recently dominating the field of oncology which is "tumors are not alike". It has been recently stated that

[*] **Corresponding authors Dr. Rana Ahmed Youness:** Pharmaceutical Biology Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, 11835, Cairo, Egypt; E-mails: rana.ahmed-youness@guc.edu.eg; rana.youness21@gmail.com, **Prof. Mohamed Zakaria Gad:** Biochemistry Department, Faculty of Pharmacy and Biotechnology, German University in Cairo, 11835, Cairo, Egypt; Tel: 002-01272222695; Fax: +202-2-2759 0711; E-mail: Mohamed.gad@guc.edu.eg

Yusuf Tutar (Ed.)

the heterogeneity among malignant tumors is refereed as one of the main obstacles leading to improper eradication of cancer until this moment [2].

Heterogeneity in solid tumors is classified into 3 main types: 1) inter-tumor heterogeneity among patients, 2) intra-tumor heterogeneity within the same tumor and 3) temporal heterogeneity in the tumor during developmental changes or changes in response to treatment [2], thus making the legend of cancer even more puzzling and challenging, and the introduction of "precision medicine" in oncology and its extrapolation to "personalized treatment code" for each cancer patient is a deep necessity.

BREAST CANCER (BC)

Breast cancer (BC) comes on top of the list of such heterogeneous solid tumors. BC comprises a multiplicity of tumor subtypes that have various treatment responses since they are demonstrated in many clinical, pathological and molecular profiles [3]. It is also hypothesized that the reason many BC patients experience resistance to conventional protocols may be due to the heterogeneity of BC [4]. Furthermore, particular BC subtypes are described as being challenging and complicated in terms of diagnosis and treatment [3]. BC is the second most common malignancy in both sexes that comes after lung cancer, according to the "Globocan" project of the International Agency for Research on Cancer (IACR) [5]. Yet, it should be a research priority as it is the most common malignancy among females [5].

INCIDENCE AND PREVALENCE

Globally, BC comprises nearly 25% of all incident cancer cases [5]. BC is notoriously known for its high incidence rate in all countries [6]. According to the latest world cancer statistics available from the IARC around 1.7 million patients were diagnosed with BC and 577,000 women died in 2012 [5]. Almost 50% of BC patients are diagnosed in low- and middle-income countries, which is a disappointment because they are already experiencing a double burden of rising non-communicable diseases with existing prevalent infectious diseases [7]. Moreover, much higher mortality rates in these countries are seen considering the incidence-to-mortality ratio, with almost 60% of BC deaths [8]. It is also worth noting that a large variation has been observed in BC survival rates around the world, with an estimated 5-year survival rate that may reach to 80% in high income countries. However, less than 40% survival rate was experienced by patients in low income countries [9]. Such terrifying statistics are definitely suggesting slow progress made in the prevention setting of such dominating disease and the prominence of BC as an endemic tribulation in our countries.

BC IS NOT JUST ONE DISEASE

Stemming from the unanimous heterogeneity of BC; BC is acknowledged for its disparate clinical behavior and patient outcomes [10]. Therefore, BC cannot be viewed as a single clinico-pathological entity, but it must be dissected into a number of more homogeneous entities known as BC subtypes. The idea that BC is not just a disease with a few variants, but a representative of diverse neoplastic diseases is also supported by the heterogeneity of BC among patients [11]. Distinct nature of such neoplastic diseases are usually realized through traditional pathological examination [12], however the actual great diversity among BC patients can be known only through molecular, proteomic and metabolic analyses as shown in Table **1** [13].

Table 1. Molecular Subtypes of BC.

Molecular Subtype	Biomarker Profile
Luminal A	ER^+ and/or PR^+, $HER\text{-}2^-$, and low Ki-67 (<14%)
Luminal B	ER^+ and/or PR^+ and $HER\text{-}2^+$ (luminal-HER2 group)
Luminal B-like	ER^+ and/or PR^+, $HER\text{-}2^-$, and high Ki-67 (≥14%)
HER-2	ER^-, PR^-, and $HER\text{-}2^+$
Triple Negative Breast Cancer (TNBC)	ER^-, PR^- and $HER\text{-}2^-$

MOLECULAR CIRCUITS UNDERLYING BC

Heterogeneity concept in BC is also supported by molecular pathogenesis studies. BC was referred to as collection of diseases with variable molecular underpinnings modulating therapeutic responses, disease-free intervals, and long-term survival of patients [10]. It is very disappointing that the molecular basis of such malignant transformation process has remained elusive and considered as one of the most challenging aspects of the disease, given all current efforts directed towards research on BC [14]. Comprehensive analysis and assessment of the molecular features of the disorder is necessary to reach a true personalized BC treatment. Additionally, understanding the impact of specific genetic and epigenetic changes and their combinations is also necessary to achieve the goal of personalized management of patient [10]. Some molecular circuits that are changed are shown in Fig. (**1**) underlie in the pathogenesis of BC such as JAK/STAT, PI3K/AKT/mTOR and RAS/RAF/MAPK signaling pathways [15]. In this figure, oncogenic signaling cascades are drawn downstream from a collection of aberrantly expressed tyrosine kinase receptors (such as insulin like growth factor-1 receptor, IGF-1R), EGFR and HER-2 receptors or cytokines receptors (such as IL-1 and TNF-α), or chemokine receptors (such as C-C

CHAPTER 6

Combining Imaging and Drug Delivery for Cancer Treatment

Seda Keleştemur[1,*] and **Gamze Yeşilay**[2]

[1] *Department of Biotechnology, University of Health Sciences-Turkey, Hamidiye Institue of Health Sciences, Selimiye Mah., 34668, Istanbul, Turkey*

[2] *Department of Molecular Biology and Genetics, University of Health Sciences-Turkey, Hamidiye Health Sciences Institue, Selimiye Mah., 34668, Istanbul, Turkey*

Abstract: Theranostics is the definition of bringing the imaging agent and therapeutic drug together in the same delivery design. The term 'theranostic' was first defined by John Funkhouser in 2002 and since then it became one of the most attractive fields in treatment of severe diseases. Nanoparticles (NPs) are the most suitable carrier systems due to their plasmonic and magnetic properties, active surface areas and various physicochemical properties. Development of therapeutic NPs provide both active and passive targeting, sensitive monitoring of biological circulation, effective drug carrying and releasing, longer circulation time and efficient clearance from renal system. Here in this chapter, we discussed commonly used cancer treatment theranostic NPs that utilize imaging modalities such as magnetic resonance imaging (MRI), radionuclide-based imaging; positron emission tomography (PET) and single-photon emission computed tomography (SPECT) and X-ray-computed tomography (CT).

Keywords: Cancer, Computed tomography, Drug delivery, Imaging, Magnetic resonance imaging, Nanoparticles, Radionuclide-based imaging, Theranostic.

INTRODUCTION

The importance of diagnosis in the treatment of severe diseases and especially in cancers is high. Therefore, efforts are accumulated on the development of smarter diagnostic systems.

An emerging branch of diagnostic approaches is the design of theranostic particles where both 'thera'py and diag'nostics' is made possible with one particle. In classical diagnosis, if we take magnetic resonance imaging as an example, a high concentration of contrast agent is given to patient to obtain higher sensitivity. By utilizing theranostics, it is aimed to deliver a therapeutic dose while the patient al-

[*] **Corresponding author Seda Keleştemur:** Department of Biotechnology, University of Health Sciences-Turkey, Hamidiye Institue of Health Sciences, Selimiye Mah., 34668, Istanbul, Turkey; E-mail: seda.kelestemur@sbu.edu.tr

Yusuf Tutar (Ed.)

ready received high amount of exogenous material and while a high spatial resolution is obtained to monitor the disease region.

Nanomaterials are a promising platform to design smart theranostic tools for their biocompatible size range, modifiable surface, high surface-to-volume ratio that can enable higher drug loading efficiency, and excellent optical and electrical properties that can enable tunable photodynamic therapy (PDT), photothermal therapy (PTT) and many other possible modalities.

Here in this chapter, it was aimed to introduce nanotheranostic approaches in the treatment of cancers by grouping into imaging modalities. Under each imaging modality, innovative nanoparticle (NP) designs were explained and examples from the recent literature were provided.

MAGNETIC RESONANCE IMAGING

Magnetic resonance imaging (MRI) is a valuable tool in soft tissue imaging together with its high spatial resolution and tissue depth-independent imaging abilities. Utilization of tissue contrasting agents provides extra sensitivity to the technique. Moreover, by modifying the contrast agents, theranostic applications of MRI is made possible alongside with tracking the payload release by measuring changes in T_1 and T_2 relaxation, and chemical exchange saturation transition (CEST) contrast in tissue of interest [1 - 3].

Current theranostic MRI applications mostly include; pH- [4, 5], hyperthermia- [6], light- [7] or ultrasound-mediated [8] chemotherapeutic drug release, PDT [9 - 11] or PTT [12, 13], siRNA-mediated gene knockdown [13, 14], microbubble generation upon laser irradiation [15] or ultrasound stimulus [8] as well as magnet-guided tissue localization of liposomes [16].

The drug and contrasting agent carrier systems can also be coated with various materials. Coating enables targeted delivery of drugs by the help of targeting moieties such as anti-EGFR [17], anti-VEGF [18], aptamers [4] or folate [19], depending on the tissue or tumor of interest. Polyethylene glycol (PEG) can be used to enhance biocompatibility of metals such as Gd^{3+}, which is used as contrasting agent for MRI [20, 21]. It is possible to increase drug-loading capacity by utilizing polymers to load contrasting agents on them [13] or to cross challenging barriers in the body such as blood-brain-barrier (BBB) by utilizing surfactants such as Tween-20 [22].

There are several approaches to obtain a final theranostic agent for MRI applications. For instance, NPs such as SPIONs are used as contrast agents and can be loaded in liposomes together with a chemotherapeutic drug [22]. Once they

reach tumor site, the drug is released whereas tissue contrast is obtained in MRI.

Liposomal delivery is actually widely investigated strategy for its ability to deliver large payloads at once. Various compositions of liposomes enable more efficient cargo delivery to the tissue of interest. Among them, long-circulating liposomes, which are mostly coated with a stealth polymer such as PEG, are promising theranostic tools. These sterically stabilized liposomes provide escape from reticuloendothelial system (RES) and increase tumor accumulation [23]. Addition of a targeting moiety to the liposome increases the tumor accumulation and minimizes non-specific tissue damage. For instance, in a study by Kwon *et al.,* anti-EGFR antibody was coated on liposome surface where the MagLipo liposomes contained magnetic iron oxide NP core-SiO_2 shell [17]. The liposomes were also loaded with doxorubicin (Dox) as a chemotherapeutic drug and siRNA against Plk1, a gene overexpressed in pancreas cancer. These pH-sensitive liposomes showed successful therapeutic effect *in vitro*. Another interesting liposomal nanotheranostic tool is self-assembling ABCD NPs. The ABCD NP concept was introduced by Kostarelos and Miller in 2005 [24], where each layer was named after each letters of ABCD. The core layer (layer A) is a nucleic acid layer such as siRNA, mRNA, or pDNA. Then comes layers B, C, and D corresponding to lipid envelope layer, stealth or biocompatibility layer, and biological recognition layer, respectively. They also suggested that NPs containing only AB layers can be used *in vitro*, whereas ABCD or ABC NPs can be utilized *in vivo*. An application of this approach can be exemplified from a study by Kenny *et al*. [14], where they designed a nanotheranostic agent against ovarian cancer on OVCAR-3 human ovarian cancer xenograft mice and they termed the particles as liposome-entrapped siRNA (LEsiRNA). The particles contained anti-Survivin siRNA entrapped in pegylated cationic liposomes and the liposome formulation contained Rhodamine and Gd-DOTA which provided bimodal imaging; fluorescent and MRI, respectively. In a similar approach, Liu *et al*. designed a multilayered particle [25]. In the core layer, there were Fe_3O_4 NPs whereas a mesoporous SiO_2 layer covered this layer to form magnetic mesoporous silica NPs (FM). The porous structure of FM was doped with Dox and the particles were entrapped in a lipid bilayer, which was loaded with zinc phtalocyanine for PDT. Finally, the bilayer was coated with PEG-Methotrexate for chemotherapeutic effect. All of these modifications provided magnet-guided localization, stimuli and pH-triggered release of drugs whereas trimodal imaging was made possible; MRI, fluorescent and photoacoustic imaging.

Polymers have been applied as drug and contrast agent carriers. Among others, poly(lactic-co-glycolic acid) (PLGA)-based systems have been widely tested *in vivo* for the theranostics of various cancers in animal models. In many cases, the polymer is loaded with perfluorocarbon or perfluorohexane gas together with a

CHAPTER 7

Practical Clinical Applications: Chemotherapy and Nuclear Medicine

Turkan Ikizceli[1,*] and **S. Karacavus**[2]

[1] *Department of Radiology, University of Health Sciences Turkey, Haseki Training and Research Hospital, Istanbul, Turkey*

[2] *Department of Nuclear Medicine, University of Health Sciences Turkey, Kayseri City Training and Research Hospital, Kayseri, Turkey*

Abstract: An optimized and particular cancer therapy must deliver the right type of treatment to the right targeted tissue to achieve control of the disease efficiently with minimal local and systemic toxicity and side effects. Advances in nanotechnology have introduced some approaches that offer new alternatives to diagnose and treat after being used in medicine. When the hydrophilic molecules are attached as carrier particles, they may remain in circulation for longer, which leads to the target organ. These new advances in recent years in nanotheranostics have expanded this concept and allowed characterization of individual tumors, prediction of nanoparticle–tumor interactions, and creation of tailor-designed new nanomedicines for individualized treatment in medicine. Advances in imaging technologies used in diseases, in general, have resulted in additional consortium guidelines for standardizing diagnostic imaging in clinical oncology. Diagnostic imaging using Ultrasonography (US), Computed Tomography (CT), Magnetic Resonance Imaging (MRI), and Positron Emission Tomography (PET) have been the most important tools. Nuclear Imaging allows a proper diagnosis, much earlier treatment, and better follow up opening a new door by non-invasive *in vitro/ex vivo* assessments in the oncology field and for personalized medicine. A nanotheranostic probe for nuclear medicine gives combined diagnostic and therapeutic capabilities by radiolabeling the different emitters (α, β^+, β^-, γ) used for imaging and/or therapy. The radiolabeled nanoparticles consist of the labeling of radionuclides onto the nanomaterials that cause deeper penetration increasing internal radiotherapy in cancer cells and inducing cell death. An ideal radionuclide nanotheranostic probe has properties such as long shelf life, easily accessible radionuclides, convenient half-life, easy and high marking efficiency, *in vivo* stability, lack of immunological reaction, rapidly clearance from circulation and directed to the target, high image quality, retention of radionuclide in the liposome and its metabolites should be non-toxic. The emergence and its further development of the nanotheranostic

[*] **Corresponding author Turkan Ikizceli:** Department of Radiology, University of Health Sciences Turkey, Haseki Training and Research Hospital, Istanbul, Turkey; E-mail: turkan.ikizceli@sbu.edu.tr

Yusuf Tutar (Ed.)

concept illustrate the need for a multidisciplinary approach with the common objective of improving the management of clinical oncology trials. The simultaneous yield of imaging in radiologic and nuclear medicine applications and therapeutic agents offer the possibility of diagnosis and treatment feedbacks on the treatment effectiveness in real-time.

Keywords: Cancer diagnosis, Cancer therapy, Chemotherapy, Computed tomography, Imaging modalities, Magnetic resonance imaging, Nanotheranostics, Nuclear medicine.

INTRODUCTION

Advances in nanotechnology have introduced some approaches that offer new alternatives in drug production, diagnosis, and treatment after being used in medicine [1]. The most crucial advantage of nanoparticles is that their particle size is very tiny. Due to their small size, the ability to easily pass through the vessels and circulation forms the basis of their usability in treatment. When the hydrophilic molecules are attached as carrier particles, they may remain longer in circulation. More than one active unit can be loaded on a carrier, and can be directed to the target organ [2]. This helps in the drug-release monitoring, imaging-guided local treatment, and post-treatment response monitoring and follow-up. Also, the progress of nanotheranostics has allowed the characterization of individual tumors, estimation between nanoparticles and intra-tumor interactions, and the formation of tailor-designed nanomedicines for individualized treatment. An optimized cancer therapy must deliver the right treatment design to the accurate target to have localized control of the disease with minimal systemic toxicity and side effects [3]. But in reality, there is significant variation among tumors and individual patients. It will require careful coordination of diagnosis and treatment, real identification of the patient and tumor subgroups, and medications. Developments in nanotechnology in medicine offer a promising opportunity for this new field and create a modern workplace. At the same time, treatment can be monitored non-invasively and in real-time. A traditional nanotheranostic agent is made by combining both diagnostic and therapeutic components [3].

Applications of Nanotheranostics in Cancer Chemotherapy

The combination of imaging and treatment using nanotechnology has become one of the forefront topics of research nowadays. In the last decade, plenty of nanoparticles have been developed for their potential applications as diagnostic and therapeutic agents. The use of nanotechnology in medicine, known as nanomedicine, has increased the interest, as a variable strategy for choosing the right drug delivery and diagnostic purposes. Nanotheranostics gives hope because

it integrates the simultaneous and non-invasive diagnosis and treatment of diseases with the exciting possibility to monitor drug release and distribution in real-time, thus giving notice and confirming the impact of the procedure. These features of nanotheranostics are very engaging for optimizing therapy results in oncological clinical trials. The next step of the trial is using them for an accurate, personalized medicine that will adapt the optimized therapy to cancer patients [4].

Clinical application of nanotheranostics makes it possible to detect and treat cancer earlier, estimate, monitor, and screen patient responses to treatment [5].

An ideal nanotheranostic agent should have the following features: a) Ability to detect tumor location, b) High signal-to-noise ratio (SNR), c) High specificity for early detection, d) Non-toxic or at least minimal systemic toxicity at the concentration necessary for the diagnostic signal. These features make it possible (1) for early detection, (2) proper drug selection, (3) discovery of biomarkers, (4) staging of the disease, (5) monitoring drug release in real-time, and (6) determination of therapeutic outcomes during therapy and post-therapy [6].

The mechanism of traditional chemotherapy seeks to exploit the differences between cancer and healthy cells. Unfortunately, chemotherapeutic drugs still have high systemic toxicity and considerable morbidity in modern medicine. Due to this systemic toxicity, tumors rapidly develop resistance and limit the dosage of these drugs. Nanotherapeutics achieve a synergistic effect by blocking multiple receptors within cancer, reducing systemic toxicity by minimizing the dose of the medicine in the circulation. Furthermore, by combining these drugs with the target molecule, they specifically help to bind the targeted chemotherapeutic agent to cancer cells and increase the therapeutic index [7]. Although the effectiveness of nanotheranostics has been proved, many are still far from clinical practices. Treatment of cancer requires an accurate diagnosis and correct staging using a specific imaging method before planning the therapy.

Imaging plays the primary role in cancer diagnosing, staging, and monitoring in clinical trials. In recent years, a considerable number of new imaging technologies and targeted tracers have been developed for cancer imaging but are not yet in clinical use due to a lack of sufficient standards [8]. Also, personalized medicine is gaining immense importance. A particular type of treatment for a specific disease may be beneficial for one individual, but not for the other patients. The different responses of patients to the same procedure mostly depend on their genetic differences. Because of this variation, it is difficult to make an accurate prediction of many medical conditions [9, 10]. With the increase in personalized therapeutic approach, diagnostic methods and imaging modalities are of great help in providing reliable and early clinical phase assessments. Thus, an ideal

SUBJECT INDEX

A

B

C

D

E

F

G

H

I

J

L

M

N

O

P

Q

R

S

T

U

V

W

X

Z

YUSUF TUTAR

Prof. Dr. Yusuf TUTAR obtained his M.Sc. and Ph.D. degrees from Oregon State University and Texas Tech University, respectively. He pursued his postdoctoral study at the National Institutes of Health (NIH/NIDDK), USA. His research focuses on biochemistry, biophysics, genetics, and molecular biology with specialization in the fields of protein structure-function, protein folding, prion, microRNA, pseudogenes, molecular cancer, proteomics, genomics, protein expression and characterization by spectroscopic and calorimetric methods. Dr. Tutar is currently continuing his research on drug design at the University of Health Sciences, Turkey. He is the head of biochemistry division at the Hamidiye Faculty of Pharmacy and the head of molecular medicine division at Hamidiye Health Science Institutes.

Dr. Tutar received 15 awards, including the National Young Scientists Award-Turkey, FEBS-EU, Teach Academy-USA, the Honor Society Membership-Texas. He is the author of 80 articles, 24 book/book-sections and EBM of Bentham Medical Books, Anticancer Agents in Medicinal Chemistry, and Medicinal Chemistry journals.