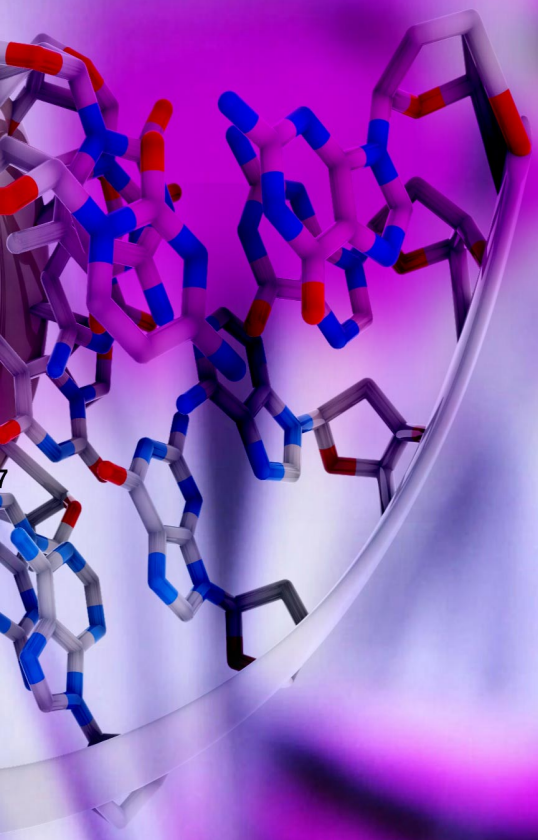
ISSN: 2213-3585
eISBN: 978-1-68108-076-5
ISBN: 978-1-68108-077-2

Volume 4

Topics in Anti-Cancer Research



Editors:
Atta-ur-Rahman, *FRS*
Khurshid Zaman

Bentham Books

Patents eBook Series
"Topics in Anti-Cancer Research"

Volume 4

Editors

Atta-ur-Rahman, *FRS*
Kings College
University of Cambridge
Cambridge
UK
&
Khurshid Zaman
Bentham Science Publishers
USA

advertisements or ideas contained in the Work.

Limitation of Liability:

In no event will Bentham Science Publishers, its staff, editors and/or authors, be liable for any damages, including, without limitation, special, incidental and/or consequential damages and/or damages for lost data and/or profits arising out of (whether directly or indirectly) the use or inability to use the Work. The entire liability of Bentham Science Publishers shall be limited to the amount actually paid by you for the Work.

General:

1. Any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims) will be governed by and construed in accordance with the laws of the U.A.E. as applied in the Emirate of Dubai. Each party agrees that the courts of the Emirate of Dubai shall have exclusive jurisdiction to settle any dispute or claim arising out of or in connection with this License Agreement or the Work (including non-contractual disputes or claims).
2. Your rights under this License Agreement will automatically terminate without notice and without the 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 Ltd.
Executive Suite Y - 2
PO Box 7917, Saif Zone
Sharjah, U.A.E.
Email: subscriptions@benthamscience.org

CONTENTS

FOREWORD

This is the fourth volume of the Series entitled ***Topics in Anti-Cancer Research*** that constitutes updated reviews on important topics relevant to recent cancer studies based on articles published in the journal *Recent Patents on Anti-Cancer Drug Discovery*. The topics included in the present volume will be of great value to clinicians, scientists and R&D experts aiming to access the latest developments in the field of cancer treatments.

There is increasing interest in antibody-delivered drugs and toxins in cancer therapeutics. This is because they selectively direct highly potent cytotoxic agents to cancer cells that present tumor-associated surface markers. This results in a reduction in systemic toxicity. In the first Chapter Dosio *et al*. present immunotoxins and antibody-drug and antibody-toxin conjugates as potential anti-cancer agents.

Enhanced expression of the multi-functional protein nucleolin (NCL) ihas been observed on the surface of activated lymphocytes, angiogenic endothelial and several different types of cancer cells. Papadimitriou *et al*. discuss how the cell surface nucleolin can act as a target for cancer therapy, thereby providing a research approach targeting NCL cancer therapy.

Tubulin is a useful and strategic molecular target for developing new anticancer drugs since the process of microtubule assembly and disassembly can be blocked by agents that bind to the β-tubulin subunit. Nepali and Ohja, in Chapter 3, have presented the latest developments in the field of tubulin (polymerization) inhibitors, modified analogs and derivatives involved in the anti-tubulin drug discovery.

Resveratrol is a natural polyphenol found in red grapes and other natural sources that has attracted much interest because of its many beneficial effects as an antioxidant, anti-inflammatory, and antiatherogenic agent, as a platelet aggregation inhibitor, as well as as an antiproliferative and proapoptotic factor in various types of cancers. Mihăilă discusses the chemosensitizing role of resveratrol as a potential drug on leukemic cells, the low bioavailability and new extraction methods and new formulas for optimizing resveratrol effects in the light of patent studies on adjuvant agents for the cure of oncohematological disorders in Chapter 4.

Cytokines and chemokines play an important role in cancer-related inflammation. In view of the multiple functions of cytokines and chemokines in tumorigenesis, There is considerable research directed at the elucidation of these roles in order to get a better understanding of the pathological processes of cancer development that can lead to innovative anti-cancer strategies. Amedei and Prisco, in Chapter 5, have reviewed the role of cytokines and

chemokines pathways in cancer immunotherapy for different cancer types.

The fusion of two or more sub-structures that serve as pharmacophoric sub-units can lead to the development of more powerful anti-cancer hybrid drugs, a process known as molecular hybridization (MH). Chapter 6 by Nepali and Mehndiratta focuses on the use of this approach for the design of anticancer hybrids. The structures of the hybrid drugs and their mechanisms of action in various cell lines are reviewed.

Gold rods, when irradiated with ultrasound, result in bio heat transfer to tissue, which can be a potential alternative to kill cancer cells. Several methods for cancer treatment with hyper-thermia are currently available. These approaches are discussed in Chapter 7 by Ioannis *et al*.

There is an inverse correlation between estrogen-levels and breast cancer risk in obese women. The underlying causes of this inverse correlation should lead to a better understanding of breast cancer etiology and promote primary prevention measures Suba in Chapter 8 summarizes the various methods reported for the prevention and therapy of obesity -related disorders and the associated breast cancer due to insufficient estrogen levels or loss and increased tendency of insulin resistance.

Talevi *et al*. in Chapter 9, present the recent applications of polymeric - based nanosystems as nanodelivry vectors for the treament of differenrt cancers. The study emphasizes the patents on polymer based organic nanocapsules, nanospheres, dendrimers and nanomicelles and their role in polymeric nano delivery systems due to their physiochemical and biochemical characteristics.

In Chapter 10, lipid based patented Nano system applications for anticancer drug delivery is discussed by Alan & Ruiz. These lipid based formulations affect the pharmacokinetics and pharmacodynamics properties of the drugs used in the treatment of cancer.

Santibanez *et al*. in Chapter 11, review the interaction between the transforming growth factor beta1 (TGF-β1) and urokinase type plasminogen activator (uPA) system in tumorigenesis. The pathways of regulation of uPA expression by TGF- β in tumor, and conversion of plasminogen to plasmin which may release TGF-β1in cancer cells, are supported by current cancer treatments and patents on TGF- β

Cheepsattayakorn, in Chapter 12, describe the functions and mechanisms of action of novel compounds and analogs discussed in recent patents for the treatment of non small cell lung cancer and small lung cancer chemotherapy.

Rinaldi *et al*. provide recent coverage of DNA vaccines against cancer involving enhance-ment of immunogenicity by the application of different approaches. The use of immune

modulators derived from tetanus and vegetable toxins and other immune molecules is reviewed in Chapter 13.

We hope that the present fourth volume will attract audience from cancer field, including oncologists, R&D scientists, as well as researchers in pharmacology and medicinal chemistry, looking for safe new methods and drugs for the inhibition & cure of cancer and for the clinical trials of new anti-cancer drugs.

The editors are grateful to the authors for their excellent and timely contributions. We also wish to thank Mr. Mahmood Alam, and Mrs. Rafia Rehan. As well as other members of Bentham Science Publishers for their active involvement and support in the completion of the eBook.

Atta-ur-Rahman,FRS
Khurshid Zaman

List of Contributors

Amedei Amedeo	Department of Experimental and Clinical Medicine, University of Florence, Largo Brambilla, 3, Firenze, 50134, Italy
Cheepsattayakorn Attapon	Department of Disease Control, Ministry of Public Health, 143 Sridornchai Road Changklan Muang, Chiang Mai, 50100, Thailand
Dosio Franco	Dipartimento di Scienza e Tecnologia del Farmaco, University of Torino, v. P. Giuria 9, Torino, I-10125 , Italy
Gkigkitzis Ioannis	Department of Mathematics, East Carolina University, 124 Austin Building, East Fifth Street, Greenville, NC , 27858-4353, USA
Mihăilă Romeo G.	Faculty of Medicine, University of Sibiu, Spitalul Clinic Județean de Urgență Sibiu, Str. Lucian Blaga, nr 2A, Sibiu, Sibiu, 550169, Romania
Nepali Kunal	ISF College of pharmacy, Moga, Punjab, India
Papadimitriou Evangelia	Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Patras, GR, 26504, Greece
Rinaldi Monica	Institute of Translational Pharmacology, National Research Council, Via Fosso del Cavaliere , Rome, 100 - 00133, Italy
Santibanez Juan F.	Laboratory for Experimental Hematology and Stem cells, Institute for Medical Research, University of Belgrade, Dr Subotića 4, Belgrade, 11129, Serbia
Suba Zsuzsanna	National Institute of Oncology, Surgical and Molecular Tumor Pathology Centre, H-1122 Ráth György str. 7-9, Budapest, Hungary
Talevi Alan	Biopharmacy, Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP) - Argentinean National Council for Scientific and Technical Research (CONICET) CCT La Plata, 47 and 115, La Plata, Buenos Aires, B1900AJI, Argentina

CHAPTER 1

Targeted Cancer Therapy: The Roles Played by Antibody-Drug and Antibody-Toxin Conjugates

Franco Dosio[1,*], Barbara Stella[1], Paola Milla[1], Carlo Della Pepa[1], Daniela Gastaldi[2], Silvia Arpicco[1]

[1] *Dipartimento di Scienza e Tecnologia del Farmaco, University of Torino, Torino, I-10125, Italy*

[2] *Dipartimento di Biotecnologie Molecolari e Scienze per la Salute, University of Torino, Torino, I-10125, Italy*

Abstract: In recent years, antibody therapeutics have been widely and successfully used in treating cancer. Antibodies that specifically bind tumor surface antigens can also be used as therapeutics, and over 35 of them are in clinical use (e.g. trastuzumab, bevacizumab and cetuximab). However, some unmodified antibodies against tumor-specific antigens lack therapeutic activity. Conjugation to cytotoxic agents can increase the antibodies' activity and, at the same time, enable extremely cytotoxic drugs to be used.

Antibody-delivered drugs and toxins are poised to become important classes of cancer therapeutics. These biopharmaceuticals have potential in this field, as they can selectively direct highly potent cytotoxic agents to cancer cells that present tumor-associated surface markers, thereby minimizing systemic toxicity. The activity of some conjugates is of particular interest receiving increasing attention, thanks to very promising clinical trial results in hematologic cancers. Over forty antibody-drug conjugates and six immunotoxins now in clinical trials, as well as some recently approved drugs, support the maturity of this approach.

This chapter focuses on recent advances in the development of these two classes of biopharmaceuticals: conventional toxins and anticancer drugs are described, together with their mechanisms of action. The processes of conjugation and purification, as reported in the literature and in several patents, are discussed and the most relevant results in clinical trials are listed. Innovative technologies and preliminary results on novel drugs and toxins, as reported in the literature and in recently-published patents (up to January 2015) are lastly examined.

[*] **Address Correspondence to this Author:** Franco Dosio. Dipartimento di Scienza e Tecnologia del Farmaco, University of Torino, v. P. Giuria 9, I-10125 Torino, Italy; Tel +390116706661, Fax +390112367697, E-mail: franco.dosio@unito.it

Atta-ur-Rahman and Khurshid Zaman (Eds)

Keywords: Alpha-amanitin, antibody-drug conjugate, anticancer agents, auristatins, calicheamicins, cancer, clinical trials, conjugation strategy, cross-linkers, diphtheria toxin, doxorubicin, duocarmycins, immunotoxin, maytansinoids, pseudomonas exotoxin, pyrrolo[1,4] benzodiazepines, ribosome inactivating proteins, SN-38, solid tumors, tubulysin.

1. INTRODUCTION

Monoclonal antibodies (mAbs) are eminently suitable as drug carriers, thanks to their selectivity and flexibility. The recent successful development of monoclonal antibodies that target key components of biological pathways has expanded the range of treatment options for patients with several cancers.

Antibody-based therapeutics are of growing significance in cancer therapy, as is shown by the fact that more than 40 formulations (in the form of a mAb as such or conjugated with a drug) have now been approved for oncologic indications by the FDA and are marketed in the USA. Eight of these had global market revenues above US $1 billion, and their combined global revenues exceed US $50 billion. These therapeutics are one of the fastest growing sectors in the pharmaceutical industry. The commercial pipeline of mAb-based therapeutics is still growing, and nearly 350 candidates [1], not only for oncological use, but also for several immunological indications and for Alzheimer's disease, are now in clinical development. Progress in the development of antibody-based therapeutics is dramatically accelerating.

Further, the traditional bivalent, monospecific, full-length IgG molecule only accounts for about half of the anticancer mAbs in the pipeline. The rest are compounds that can be conjugated to drugs, toxins or radiolabels; they may be multispecific or otherwise engineered for increased functionality [2]. Immunoconjugates have evolved over 40 years; with antibody-drug conjugates (ADCs) they recently reached significant clinical and regulatory milestones, with the marketing approval of brentuximab vedotin. Molecular engineering techniques have also had a significant impact on the efficacy/toxicity profiles of immunotoxins (ITs), for example denileukin diftitox, which is now registered for the treatment of cutaneous T-cell lymphomas.

This chapter will provide an overview of the more advanced ADCs and ITs in the industrial pipeline, or under development in academic research laboratories, updated to the end of 2014. The mechanisms underlying cell intoxication by toxins or by anticancer drugs will first be presented, followed by the evolution of the structure of conjugates obtained by molecular biology or chemical synthesis. On the basis of the targeting agent employed, the conjugated will be divided into two major groups: those targeting hematologic and those targeting solid cancers. Finally, the most advanced results obtained in preclinical or clinical trials will be described in some detail. Due to the impressive body of basic research in the field of ADCs and ITs, the chapter will concentrate chiefly on patented approaches.

Immunotoxins are protein-based therapeutics consisting of a targeting moiety linked or fused to a killing moiety. The target moiety can be an antibody or a ligand directed against a receptor or cell-surface antigen that is specific for the targeted disease, while the active moiety is a member of a class of highly toxic proteins or enzymes. Essentially, any molecule that induces cell death by directly interfering with the cell machinery, by modifying the cell membrane, or by inducing apoptotic proteins can be used. Because of the enzymatic potency of these proteins, a small number of toxin molecules successfully delivered to the cytoplasm (or to the ribosomal compartment) may be lethal to the cell [3].

Initially, ITs comprised mAb or growth factors chemically conjugated to cytotoxic plant or bacterial toxins [4], but these have largely been replaced by recombinant methods, thanks to their considerable design flexibility and product homogeneity.

Monoclonal antibody technology is now mature, fully humanized mAbs are in therapeutic use, and fragments of varying complexity are available. Thus the most strenuous and continuous efforts are directed to manipulating the killing moiety, where the success of delivering the cytotoxic domain of an IT to the cell cytoplasm depends on a series of steps, each having varying degrees of efficiency depending on the cell type, antigen density, binding affinity, internalization/ recycling, and subcellular trafficking or endosomal escape. High anticancer activity is unfortunately usually contrasted by critical drawbacks, which are caused by varying degrees of nonspecific toxicity, mainly affecting hepatocytes,

CHAPTER 2

Targeting Cell Surface Nucleolin in Cancer

Marina Koutsioumpa[#], Effrosyni Choleva , Evangelia Papadimitriou[*]

Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, Patras GR26504, Greece

Abstract: Enhanced expression of the multi-functional protein nucleolin (NCL) is observed on the surface of activated lymphocytes, angiogenic endothelial and many different types of cancer cells. Translocation of NCL at the external side of the plasma membrane occurs via a secretory pathway independent of the endoplasmic reticulum-Golgi complex, requires intracellular intact actin cytoskeleton, and seems to be mediated by a variety of factors. Cell surface NCL serves as a binding partner of several molecules implicated in cell differentiation, adhesion and leukocyte trafficking, inflammation, angiogenesis and tumor development, mediating their biological activities and in some cases, leading to their internalization. Accumulating evidence validates cell surface NCL as a strategic target for treatment of cancer, while its property of tumor-specific uptake of targeted ligands seems to be useful for the development of non-invasive imaging tools for the diagnosis of cancer and for the targeted release of chemotherapeutic drugs. This chapter summarizes papers and patents related to the redistribution and the biological functions of cell surface NCL, with emphasis on the potential importance and advantages of developing efficient anti-cell surface NCL strategies.

Keywords: Angiogenesis , aptamers, cancer, cell surface nucleolin, colorectal cancer, conjugated chemotherapeutic drugs, endostatin, erbB tyrosine kinase receptors, esophageal squamous cell carcinoma, glioblastoma, growth factors, hepatocellular carcinoma, ligand specific internalization, papillary thyroid carcinoma, pleiotrophin, pseudopeptides, receptor protein tyrosine phosphatase beta/zeta, targeted delivery approaches, tumor-homing peptide F3, vascular endothelial growth factor.

[*] **Address correspondence to this author:** At the Laboratory of Molecular Pharmacology, Department of Pharmacy, University of Patras, GR 26504 Patras, Greece; Tel: +30-2610-962336; E-mail: epapad@upatras.gr

[#] Current address: Center for Systems Biomedicine, Division of Digestive Diseases, David Geffen School of Medicine, University of California Los Angeles, Los Angeles, CA, USA. Tel: 310-825-6263; E-mail: mkoutsioumpa@mednet.ucla.edu

Atta-ur-Rahman and Khurshid Zaman (Eds)

INTRODUCTION

Nucleolin (NCL) is an abundant protein of exponentially growing cells that is mainly located at the dense fibrillar and granular regions of the nucleolus [1 - 5]. It represents a particularly important multi-functional protein of the eukaryotic cell, controlling various aspects of DNA and RNA metabolism, chromatin structure, rDNA transcription, rRNA maturation and ribosome biogenesis. It was firstly identified in Chinese hamster ovary (CHO) cells and Novikoff hepatoma cells [4, 6] and was initially named protein C23 [6]. Its stability relates to the phosphorylation status of the molecule [7 - 9] and is increased in proliferating cells by inhibition of its self-cleaving activity [10]. Modifications such as phosphorylation [11 - 18], auto-degradation [10, 19] and glycosylation [20] have been linked to NCL localization in the cell and the regulation of its biological activities.

Although mammalian NCL, consisting of 707 amino acids, has a predicted molecular mass of approximately 77 kDa [21, 22], the apparent molecular mass is between 100 and 110 kDa due to the high content of negatively charged amino acids in the amino-terminal domain [23 - 25]. Analysis of NCL cDNA has revealed the presence of three major domains [21, 22]. The amino-terminal portion consists of highly acidic areas [26] and sites for phosphorylation by cell division control protein 2 homolog (CDC2), casein kinase 2 (CK2) and protein kinase C (PKC) [13, 14]. It interacts with chromatin [27, 28] and untranslated regions (UTRs) [29, 30], thus regulating rDNA transcription [31 - 33]. The central region contains four RNA-binding domains (RBDs) [34]. Nucleolar accumulation of NCL requires at least two RBDs along with its nuclear localization signal (NLS) [35]. The carboxy-terminal region called GAR or RGG domain is rich in Arg-Gly-Gly repeats and interspersed with several aromatic amino acids, such as dimethylarginine [36] and phenylalanine [37]. The RGG domain appears to be involved in NCL interactions with nucleic acids [38], as well as ribosomal [39] and other proteins, including urokinase-type plasminogen activator (uPA) [40] and its receptor uPAR [41], midkine (MK) [42, 43] and lactoferrin [44].

The interaction of NCL with ribosomal proteins [39] and specific pre-rRNA sequences [34, 45], and its implication in the pre-rRNA maturation process [46],

underlines its important role in ribosome assembly. NCL deficiency affects both the shape and the structure of nucleolus and its amount similarly affects both the proliferation rate and the rDNA transcription, supporting the notion that the function of NCL in ribosomal synthesis could be largely responsible for its effect on cell proliferation [47]. Based on the characteristic shuttling property of NCL [48], several findings suggest that it acts as a carrier of ribosomal proteins between the cytoplasm and the nucleus [48 - 50].

NCL is also involved in regulation of cell cycle and transcription. The amount of NCL is low in serum-deprived cells, whereas its expression is induced in mid and late G1 phase, demonstrating the necessity of NCL for cell cycle progression [51]. Down-regulation of NCL by siRNA in human cells results in growth arrest and defective centrosome dupli-cation, and appears to affect the integrity of the mitotic spindle, indicating its role in cell cycle regulation and cytokinesis [52 - 55]. Treatment with high concentrations of extracellular ADP down-regulates NCL protein levels in HUVEC, resulting in cell death [56]. NCL seems to be also involved in the mammalian target of rapamycin complex 1 pathway that regulates cell growth and proliferation [57], and has been shown to interact with the small dual-specificity phosphatase DUSP3, which is over-expressed in human cervical cancer cells, localizes preferentially to the nucleus, and plays a key role in cellular proliferation and senescence triggering [58]. Cytosolic retention of NCL by G_0/G_1 switch gene 2 has been associated with the maintenance of quiescence in hematopoietic stem cells and inhibition of leukemic cell proliferation [59, 60].

Concerning transcription, data are conflicting. NCL functions as a transcriptional repressor of c-Myb [61], c-Myc [62], p53 [63], alpha-1 acid glycoprotein [64], retinoic acid receptor β_2 ($RAR\beta_2$) [65] and activator protein-1 dependent trans-activation of matrix metalloproteinase-13 [66]. Conversely, NCL positively regulates matrix metallo-proteinase-9 [67], interleukin-9 [68], vascular endothelial growth factor (VEGF) [69, 70] and cyclin D1 expression [71]. It has been proposed that *CCND1* transcriptional activation in mantle lymphoma cells, that plays a crucial role in malignancy development, relates to the repositioning of *CCND1* allele in the NCL-abundant environment found in perinucleolar areas [72].

CHAPTER 3

Anticancer Agents Targeting Tubulin

Ritu Ojha[a], Sahil Sharma[a], Kunal Nepali[a,*]

[a] *Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Moga, Punjab, India*

Abstract: Molecular hybridization (MH) is a strategy of rational design of such ligands or prototypes based on the recognition of pharmacophoric sub-units in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-units, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates. The concept of molecular hybridization and the promises/challenges associated with these hybrid molecules along with recent advances in anticancer hybrids and critical discussions on the future aspects of the hybrid drugs have already been presented through number of reports. However, this chapter presents the structures of potent hybrids reported during the last two decades along with a detailed account of the patent literature from the year 1990 to 2014. Significant number of patents on the molecules designed through this valuable drug design technique clearly highlight the present focus of the researchers all around the globe towards hybrid molecules capable of amplifying the effect of individual functionalities through action on another bio target or to interact with multiple targets as one single molecule lowering the risk of drug-drug interactions and minimizing the drug resistance.

Keywords: Cancer, chalcones, colchicine, combretastatin A-4, cryptophycins, discodermolide, dolastatin, epothilones, halichondrin, hemiasterlins, 2-methoxye--tradiol, microtubules, patents, phenstatin, podophyllotoxin, steganacin, taxol®, tubulin, tumor, vinca alkaloids.

1. INTRODUCTION

Among the current targets for chemotherapy, alongside DNA, microtubules represent one of the targets for chemotherapy [1]. Microtubules play critical role in a number of cellular functions, such as chromosome segregation during cell

* **Address correspondence to Dr. Kunal Nepali:** Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Moga, Punjab, India, Tel:+ 91-8146096564; Email: Kunal_8855@rediffmail.com

Atta-ur-Rahman and Khurshid Zaman (Eds)

division, intracellular transport, cell motility, and the maintenance of cell shape [2].

Microtubules are long hollow cylindrical structures of 240Å diameter which are, with actin microfilaments and intermediate filaments, part of the cytoskeleton and found in almost all eukaryotic cells. Tubulin is the main constituent of microtubules and each tubulin monomer is composed of approximately 440 amino acids i.e. 50kDa which forms a core of two β-sheets surrounded by α-helices. Tubulin is a slightly acidic protein which occurs in a cell as α-tubulin, β-tubulin, γ-tubulin, d-tubulin, e-tubulin, z-tubulin and h-tubulin. Although most eukaryotic cells express multiple ab-tubulin (heterodimeric proteins) that can directly influence microtubule structure and function, some (but intriguingly not all) eukaryotes encode several other tubulin proteins i.e. γ, δ, ε, z and η tubulin. αβ-tubulin is the basic building block of microtubules, whereas, γ-tubulin is approximately 30% identical to α- and β-tubulin, has subsequently been described in a wide variety of eukaryotic organisms, and is located in centrosomes and other MTOCs, such as the spindle pole body (SPB) of fungi, where it plays an essential role in the nucleation of microtubule assembly; δ-tubulin has been identified in humans, mice, rats, trypanosomes and in other protists, such as the malarial parasite *Plasmodium*, and is localized to the centrosome or basal body and other regions, and exhibit basal-body defects - doublet rather than triplet arrangement of microtubules owing to specific loss of C tubule; ε-tubulin has been identified in mammalian cells and in trypanosomes, and is localized to the centrosome in cell-cycle-dependent manner, the role of ε-tubulin is at present unresolved but it is not involved in microtubule nucleation; z-tubulin has been identified in trypanosomes and in some other organisms, and is localized to the basal body region in trypanosomes and at the centriolar region in some animal cells, the role of z-tubulin is unresolved, so far; η-tubulin has been identified in *Paramecium* and has no localization data available, so far. *Paramecium* z-tubulin mutants show rare defects in basal bodies - lacking some microtubules from basal body triplets [3].

α-Tubulin and β-tubulin both have a molecule of GDP attached to them and are capable of forming dimers. These dimers attach to each other and arrange head-to-tail at 80Å intervals, to form a linear protofilament. Thirteen of these protofilaments constitute a tube like structure called microtubule [4 - 7].

Microtubules are extremely important in the process of mitosis, during which the duplicated chromosomes of a cell are separated into two identical sets before cleavage of the cell into two daughter cells. Their importance in mitosis during cell division and in essential interphase cellular mechanisms contributing to tumorigenesis or cancer progression makes them an impotant target for anticancer drugs. Microtubules and their dynamics are the targets of a chemically diverse group of antimitotic drugs that have been used with great success in the treatment of cancer. In view of the success of this class, it seems likely that drugs of this class will continue to be important chemotherapeutic agents, even as more selective approaches are developed. Microtubules also seem to be a favorite target of naturally occurring, presumably self-protective, toxic molecules that are produced by a large number of plants and animals, ranging from algae to sea hares. Most of the microtubule targeting compounds have been discovered in large-scale screens of natural products [8 - 10].

The dynamic process of microtubule assembly and disassembly can be blocked by various agents that bind to distinct sites in the β-tubulin subunit. By interfering with microtubule function, these agents arrest cells in mitosis as well as inhibit interphase functions, eventually leading to cell death, by both apoptosis and necrosis. So far, four binding domains have been identified [11 - 13]:

a) Colchicine site close to the α/β interface - Colchicine binds to a site near the intra-dimer interface and alters lateral contacts within the microtubule, blocking microtubule polymerization [14]. Agents that bind in the colchicine binding site of tubulin and inhibit cancer cell proliferation includes phenstatin (1), combretastatin A-4 (2), colchicine (3), steganacin (4), podophyllotoxin (5) and certain other synthetic analogs of these compounds (Fig. (**1**)). The two phenyl rings with appropriate substitution maintain a fixed distance between two centers of the aryl rings, which is the reason for these unconnected and structurally diverse structures to share the same activity as tubulin polymerization inhibitors. The chemical agents namely benzophenones, combretastatins, chalcones and lignans have one, two and four carbon atoms between the two-phenyl rings respectively. From the molecular models, the two aromatic rings in these molecules are arranged like the two wings of a butterfly having certain dihedral angle between them. Therefore a "Butterfly Model" with the two aromatic rings as the two wings of a butterfly is

CHAPTER 4

Resveratrol in Malignant Hemopathies

Romeo G. Mihăilă*

"Lucian Blaga" University of Sibiu, Faculty of Medicine, Emergency County Clinical Hospital Sibiu, Sibiu, Romania

Abstract: Resveratrol is a polyphenol with many beneficial effects: not only as an antioxidant, anti-inflammatory, and antiatherogenic agent, as well as a platelet aggregation inhibitor, but also as an antiproliferative and proapoptotic factor in various types of cancers. There are reviews about the mechanisms responsible for its effects in leukemia and lymphomas, emphasizing the chemosensitizing role of resveratrol, which allows overcoming the multidrug resistance of cancers. The action of resveratrol occurs preferentially on leukemic cells, and not on the normal ones. In addition, it is one of the few drugs that act on leukemic stem cells. If experimental results are promising, its application in humans encounters some difficulties. The paper presents the causes of its low bioavailability, as well as recent patents that allow improvement of its bioavailability, development of new extraction procedures, obtaining new formulae, and associating resveratrol with other drugs in order to increase its effects. These patents allow optimizing its effects in order to obtain an adjuvant agent for treatment of oncohematological disorders.

Keywords: Antioxidant, apoptosis, cell cycle, cytochrome P450, glutation, hormetic dose response, leukemia, lipoperoxidation, lymphoma, minimal residual disease, mitochondrial superoxide, multidrug resistance, multiple myeloma, nuclear factor kB, proliferation, reactive oxygen species, resveratrol, sirtuin, stem cell, tyrosine kinase.

1. INTRODUCTION

Research on the pathogenetic mechanisms involved in the appearance and development of oncohematological disorders has progressed so much that oncohematology may be a positive example for other medical specialties. The

* **Corresponding Author Lucian Blaga:** University of Sibiu, Faculty of Medicine, Spitalul Clinic Județean de Urgență Sibiu, str Lucian Blaga, nr 2A, Sibiu, 550169 Sibiu, Romania; Tel: 0040 726 340655; Fax: 0040 269 218365; E-mail: romeomihaila@yahoo.com

Atta-ur-Rahman and Khurshid Zaman (Eds)

results have been used for establishing therapeutic targets. Despite these developments, not all patients obtain complete remission, and some of them relapse. Resveratrol is a poly-phenol with many beneficial effects. The French paradox refers to a relatively low prevalence of coronary heart disease, despite a diet which includes relatively many saturated fats [1]. An explanation for this could be the relatively high consumption of red wine by the French, which contains resveratrol [2]. An experimental study on rabbits fed a hypercholesterolemic diet and treated with resveratrol showed that this drug has anti-inflammatory (low levels of monocyte chemotactic protein-1 and interleukin-6 concentrations) and anti-atherogenic effects [3]. It has lipid-lowering function and calorie-restriction effect, so that it can control, at the same time, different pathogenetic mechanisms associated with obesity [4]. The drug can improve glucose metabolism both in skeletal muscle and liver, and contribute to a better glucose control in swine with metabolic syndrome [5]. It also has positive effects on type 2 diabetes, coronary heart disease [6 - 9], neurogenerative pathologies [7] and cancer [8]. Further beneficial actions [10] of resveratrol (3,4',5-trihydroxy-trans-stilbene) have been proved, such as its antioxidant effects [5], aging prevention, and those on coagulation (it inhibits platelets aggregation) [11]. It was shown that it can modulate components of the insulin-like growth factor system in a study conducted in the plasma of volunteers [12]. Furthermore, resveratrol is a promising drug with anti-mutagenic and anti-carcinogenic proprieties [2, 9], active even in some types of oncohematological diseases resistant to drugs commonly recommended, by overcoming the mechanisms of chemoresistance [2], but it exhibits poor bioavailability in humans [13]. It is good to mention that resveratrol has inhibitory action in all three stages of oncogenesis: initiation, promotion and progression [14]. A review on the antileukemic effects of resveratrol [15] was published in 2002, followed by another in 2010. The latter focused on the possible application of resveratrol in the prevention and treatment of hematologic malignancies [16]. Another review synthesized the effects of resveratrol at cellular, molecular or physiological levels, in experimental models, in 2014 [17].

The three studies emphasized the advantages and limitations of resveratrol, and pointed to possible ways of overcoming the limitations. Since then, further

insights in the mechanisms of resveratrol have been developed, results of new studies have emerged, and new inventions have been patented in order to increase its bioavailability, as well as its antileukemic and antilymphomatous efficiency, which we intend to present in this chapter.

2. MECHANISMS OF ACTION

Origin and Effects

Resveratrol can be found in natural sources as: vegetables, grapes and red wine, cranberry, peanut, blueberry, mulberry, pine, spruce, etc. Stilbene resveratrol is a plant compound with multiple biological effects, among which are antileukemic and antilymphomatous effects. Resveratrol can sensitize the leukemic and lymphomatous cells to chemotherapy by modulating the chemoresistance pathways. It is able to overcome tumor chemoresistance by inducing apoptosis, down-regulating some drug transporters and diminishing tumor cell proliferation [2]. It has a lot of intracellular targets, such as: tumor suppressor proteins, cyclins, and other cell cycle regulators, factors involved in transcriptional pathways, regulators of cell survival and apoptosis, cyclooxygenases, nuclear factor kB (NF-kB), signal transducer and activator of transcription 3 (STAT3) pathway, etc. [2, 18]. It triggers antiproliferative, proapoptotic and anti-inflammatory effects [19]. All these considered, stilbene resveratrol can be a promising candidate for leukemia treatment [16].

Cell Penetration and Location

The process of resveratrol endocytosis in leukemia U937 cells is monensin-sensitive. Disruption of plasma membrane lipid rafts, induced by drugs such as nystatin or methyl-β-cyclodextrin, can prevent resveratrol uptake. Resveratrol can be found in sphingomyelin- and cholesterol-enriched cell fractions, where it promotes the recruitment of different signaling molecules responsible for signaling pathways. Lipid raft integrity is required for good development of the signaling process and caspase-dependent apoptosis [20].

Figure **1** and Table **1** present signal transduction pathways.

CHAPTER 5

Cancer Immunotherapy: The Share of Cytokines and Chemokines

Amedeo Amedei*, Domenico Prisco^

^ *Department of Experimental and Clinical, University of Florence & Department of Neuro-Skeletal Muscle and Sensory Organs, Interdisciplinary Internal Medicine Unit, Azienda Ospedaliero-Universitaria Careggi, Florence, Italy*

Abstract: The response of the body to cancer is not a unique mechanism and has many parallels with inflammation and wound healing. Unresolved inflammation generates a microenvironment favorable for cellular transformation and the growth of cancer cells. Chronic tissue damage triggers a repair response that includes the production of growth factors, cytokines and chemokines. In particular, cytokines and chemokines have a crucial role in cancer-related inflammation with consequent direct and indirect effects on the proliferative and invasive properties of tumor cells. In view of the multifactorial functions of cytokines and chemokines in tumorigenesis, the elucidation of their roles will further advance our understanding of the pathological processes of cancer development and highlights potential innovative anti-cancer strategies.

Despite recent advances, the main anti-cancer therapies, namely surgery, radiation therapy and chemotherapy, are limited in their ability to treat minimal and metastatic residual disease. Furthermore, the benefit of conventional therapies is often limited by collateral damage to normal tissues. Immunotherapy is a new opportunity of cancer treatment being investigated by researchers and clinicians for different cancer types.

The aim of this chapter is to analyze the recent patents and scientific reviews on the major cytokine/chemokine pathways involved in cancer immunotherapy and discuss their basic biology, clinical relevance and potential directions for future anti-cancer therapeutic applications.

Keywords: Cancer, Chemokines, CCR7, CCL2, Clinical trials, Cytokines, GM-CSF, Immunotherapy, Inflammation, Interferon-α, Interferon-β, Interferon-γ, IFN-λ, IL-2, IL-7 IL-15 IL-21 IL-6, IL-12, IL-18, IL-18.

* **Address correspondence to this author:** Department of Experimental and Clinical Medicine University of Florence Largo Brambilla, 350134 Firenze Tel/Fax: + 39 055 2758330; Email: aamedei@unifi.it

Atta-ur-Rahman and Khurshid Zaman (Eds)

INTRODUCTION

The anti-cancer response of the body is not a unique mechanism but has many parallels with inflammation and wound healing. The last century Virchow's [1] observation of the tight association between cancer and inflammation anticipated the current interest in role of immune response in tumor genesis and progression. Recent insights into the dynamics of the tumor microenvironment have begun to clarify the mechanisms underlying tumor-promoting inflammation, which bears striking similarities to wounds that fail to heal [2, 3].

Approximately 20% of cancer deaths worldwide is currently linked to unresolved inflammation [4, 5] and cancer is linked to inflammation by two pathways: extrinsic pathways from conditions that cause non-resolving smoldering inflammatory responses, and intrinsic pathways driven by oncogenes (or tumor suppressor gen-es) that activate the expression of inflammation-related programs [1].

Unresolved inflammation generates a microenvironment favorable for cellular transformation and the malignant evolution of cancer cells. Chronic tissue damage triggers a repair response that includes the production of growth factors, cytokines and chemokines [1]. Moreover, inflammatory responses consist of leukocyte cancer infiltration, neo-vascularization and fibrosis.

Cytokines and chemokines were initially identified as inflammatory mediators able to regulate the trafficking of leukocytes. Subsequent investigations revealed that chemokines also have profound effects on endothelial cells and fibroblasts, playing in this way crucial roles in cancer-related inflammation, thus affecting the major components of the inflammatory response, namely leukocytes, endothelial cells, and fibroblasts [6, 7]. Moreover, cytokines and chemokines have direct effects on the proliferative and invasive properties of tumor cells.

In other words, cytokines/chemokines have a two-faced role in carcinogenesis: they can be involved in the activation of immune effector mechanisms and so, contrasting the growth of the tumor, but at the same time, they may be involved in the different phases of carcinogenesis (tumor growth, invasion, and metastasis).

In view of the multi-factorial roles of cytokines and chemokines in tumor genesis, the elucidation of their roles will further advance our understanding of the pathological processes of tumor development and, importantly, work out pioneering anti-cancer therapeutic strategies.

To date, despite recent advances, the mainstream cancer therapies, namely surgery, radiation therapy and chemotherapy, with some exceptions, are limited in their ability to treat metastasis and minimal residual disease to cure the patients. Furthermore, the benefit of these conventional therapies is often limited by serious side effects to normal tissues. So, immunotherapy is investigated by researchers and clinicians as an encouraging option for the goal of inducing effective and long-lasting therapeutic outcome for different cancer types. It is now understood that the immune system is capable of recognizing and eliminating the cancer cells, but that tumors evade and suppress host immune responses and thus persist and spread [8, 9].

A history of cancer immune-surveillance has recently been stylishly presented by Dunn [10]. Many caveats existed in the earliest experiments in mice designed to test this hypothesis, but despite early skepticism, major progress in tumor immunology has ensued since the 1980s. Importantly, the molecular biology era brought with it the discovery of new cytokines such as interleukin-2 (IL-2) and chemokines that could activate and direct leukocytes in a coordinated fashion. Clinical applications soon followed, and chemokines /cytokine immunotherapies heralded promise of a new wave of cancer treatment [11].

The aim of this chapter is to analyze the major cytokines/chemokines involved in cancer immunotherapy and discuss their basic biology, clinical relevance and prospective directions for innovative anti-cancer therapeutic applications.

CYTOKINES: GENERAL PROPERTIES AND ROLE IN CANCER IMMUNOTHERAPY

The cytokines are a large family of molecules that are classified in various different ways due to an absence of a unified classification system. The term "cytokine" is derived from a combination of two Greek words - "cyto" meaning cell and "kinos" meaning movement. Cytokines are cell signaling molecules that aid

CHAPTER 6

Molecular Hybrids with Anticancer Activity

Samir Mehndiratta, Sahil Sharma, Sunil Kumar, Kunal Nepali*

Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Moga, Punjab, India

Abstract: Molecular hybridization (MH) is a strategy of rational design of such ligands or prototypes based on the recognition of pharmacophoric sub-units in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-units, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates .The concept of molecular hybridization and the promises/challenges associated with these hybrid molecules along with recent advances in anticancer hybrids and critical discussions on the future aspects of the hybrid drugs have already been presented through number of reports. However, this chapter presents the structures of potent hybrids reported during the last two decades along with a detailed account of the patent literature from the year 1990 to 2014. Significant number of patents on the molecules designed through this valuable drug design technique clearly highlight the present focus of the researchers all around the globe towards hybrid molecules capable of amplifying the effect of individual functionalities through action on another bio target or to interact with multiple targets as one single molecule lowering the risk of drug-drug interactions and minimizing the drug resistance.

Keywords: Activity, anticancer, chalcone, colchicine, combretasatin, coumarin, design, drug, hybrids, isatin, microtubule, molecules, patent, peptide, phenstatin, resistance, steroid, taxol, tubulin, vinca alkaloids.

1. INTRODUCTION

Molecular hybridization (MH) is a strategy of rational design of such ligands or prototypes based on the recognition of pharmacophoric sub-units in the molecular structure of two or more known bioactive derivatives which, through the adequate fusion of these sub-units, lead to the design of new hybrid architectures that maintain pre-selected characteristics of the original templates. [1, 2] It is a new

* **Address correspondence to Dr. Kunal Nepali:** Department of Pharmaceutical Chemistry, ISF College of Pharmacy, Moga, Punjab, India; Email: Kunal_8855@rediffmail.com

Atta-ur-Rahman and Khurshid Zaman (Eds)

concept in drug design and development to produce a new hybrid compound with improved affinity and efficacy, when compared to the parent drugs. Pharmacophore hybridization is believed to be analogous to conventional combination therapy, with the exception that the two drugs are covalently linked and available as a single entity [3]. The selection of the two principles in the dual drugs is usually based on their observed (or anticipated) synergistic or additive pharmacological activities to enable the identification of highly active novel chemical entities. Hybrid drugs are basically designed to counterbalance the known side effects associated with the other hybrid part or to amplify its effect through action on another bio target or to interact with multiple targets as one single molecule [4, 5] lowering the risk of drug-drug interactions and minimizing the drug resistance [1].

Overall, the scope of molecular hybridization is quite broad as it can result in compounds with modified selectivity profile, different and/or dual modes of action and reduced side effects. There is no doubt that targeting a single receptor/ enzyme/ protein is the primary strategy for the medicinal chemist but it is also assumed that the single target approach is basically the reason for the lack of successful treatment of multifactorial, complex diseases such as cancer [6 - 10]. Although the present article clearly highlights the interesting and promising anticancer profile of the hybrid structures, there are some limitations associated with these hybrid drugs which pose some serious challenges to the chemist such as high lipophilicity and chemical stability of the hybrids. In order to avoid incompatibilities, the functionalities selected for the fusion must be subjected to preliminary combination therapy. The hybrids designed by the fusion of chemical entities must outshine the cell killing potential of the individual functionalities and should also outweigh the disadvantages (compromised dose flexibility and schedule flexibility) [11].

Over the years, the researchers have exploited this technique to discover some promising chemical architectures displaying significant anticancer profiles. Molecular hybridization as a tool has been particularly utilized for targeting tubulin protein as exemplified through the number of research papers. The microtubule inhibitors such as taxol, colchicine, chalcones, and combretasatin, phenstatins and vinca alkaloids have been utilized as one of the functionality of

the hybrids and promising results have been obtained in most of the cases with some of the tubulin based hybrids exhibiting anticancer activity at nanomolar level. Linkage with steroids as biological carrier vector for anticancer drugs and the inclusion of pyrrolo [2, 1-c][1, 4] benzodiazepines (PBDs), a family of DNA interactive antitumor antibiotics derived from *Streptomyces* species in hybrid structure based drug design has also emerged as a potential strategy. Various heteroaryl based hybrids in particular isatin and coumarins have also been designed and reported to posses' remarkable inhibitory potential.

The concept of molecular hybridization and the promises /challenges associated with these hybrid molecules along with recent advances on anticancer hybrids and critical discussions on the future aspects of the hybrid drugs have already been presented through number of reports. However, this chapter presents the structures of potent hybrids reported during the last two decades along with a detailed account of the patent literature.

The anticancer hybrids have been classified into several categories on the basis of one of the functionality of the hybrids (Fig. (**1**)).

1. Tubulin Inhibitors Based Anticancer Hybrids

Microtubules are extremely important in the process of mitosis, during which the duplicated chromosomes of a cell separated into two identical sets before cleavage of the cell into two daughter cells. Their importance in mitosis and cell division makes them an important target for anticancer drugs. Microtubules seem to be the favorite target of naturally occurring, presumably self-protective, toxic molecules that are produced by a large number of plants and animals, ranging from algae to sea hares and most microtubule- targeted compounds have been discovered in large-scale screens of natural products [12 - 14].

The dynamic process of microtubule assembly and disassembly can be blocked by various agents that bind to distinct sites in the β-tubulin subunit. By interfering with microtubule function *in vitro,* these agents arrest cells in mitosis, eventually leading to cell death, by both apoptosis and necrosis. So far, three binding domains have been identified [15]: a) Colchicine site close to the α/β interface - Colchicine binds to a site near the intra-dimer interface and alters lateral contacts

CHAPTER 7

On the Use of Gold Macro-Rods and Ultrasound as a Hyperthermia Cancer Treatment: Experimental Results on Ehrlich Tumor in *Mus musculus* Mice

Carlos Austerlitz[1,†], Andre L. Barros[2,†], Ioannis Gkigkitzis[3,†*], Diana Campos[4,†], Teresinha G. Silva[5], Silene C. Nascimento[5], Ioannis Haranas[6]

[1] *Southern Illinois University, Graduate School, 1263 Lincoln Dr, Carbondale, Illinois 62901, USA*

[2] *Bioassays Laboratory for Pharmaceutical Research, Antibiotics Department, Biological Sciences Center, Federal University of Pernambuco, Recife, Brazil*

[3] *Department of Mathematics, East Carolina University, 124 Austin Building, East Fifth Street, Greenville, NC 27858-4353, USA*

[4] *Clínica Diana Campos, Recife, PE 52020-030, Brazil*

[5] *Bioassays Laboratory for Pharmaceutical Research, Antibiotics Department, Biological Sciences Center, Federal University of Pernambuco, Recife, Brazil*

[6] *Department of Physics and Computer Science, Wilfrid Laurier University, 4700 Keele Street, Toronto, ON, M3J 1P3, Canada*

Abstract: The Ehrlich tumor, derived from a mouse adenocarcinoma, has been used to investigate the bio-heat transfer and the effect of a gold macro-rod inserted into an Ehrlich tumor in white *Mus musculus* mice when irradiated with ultrasound. The *in vitro* measurements show that gold rods, when irradiated with ultrasound, not only confirm the bio heat transfer to tissue, as predicted by analytical calculations and *in vitro* measurements, but also prove to be a potential alternative to kill cancer cells. Several methods and apparatuses for cancer treatment with hyperthermia have been invented and are currently available. Out of the more than two hundred recent patents in the fields of hyperthermia and ultrasound, we mention some of them (at the introduction) that may relate to our proposed current cancer treatment methodology in future studies.

[†] These authors contributed equally to this work.

[*] **Author to whom correspondence should be addressed;** Ioannis Gkigkitzis, Greenville, North Carolina, 27858, USA; E-Mail: gkigkitzisi@ecu.edu, Tel.: +1-252-737-1554; Fax: +1-252-328-6414.

Atta-ur-Rahman and Khurshid Zaman (Eds)

Keywords: Adenocarcinoma, bio-heat, cancer, Ehrlich, frequency, gold, heat, hyperthermia, *Mus musculus*, macro-rods, nanoparticles, proteins, seed, temperature, transducer, transfer, tissue, tumor, ultrasound, xylazine.

1. INTRODUCTION

Hyperthermia is suitable to induce both necrotic and programmed cell-death in a temperature dependent manner [1, 2]. Although not yet elucidated, hyperthermia initiates cell death through the intrinsic pathway of apoptosis, and it is known to involve the transmission of the temperature elevation signal to the mitochondrion through proteins belonging to the Bcl-2 family. Preclinical developments show the importance of heat shock proteins (Hsps) and other proteins interfering and regulating the intrinsic and extrinsic pathways of apoptosis. The complexity of heat-induced changes in cells and tissues, the temperature needed, and the thermal dose sufficient for hyperthermia are still unresolved and heavily discussed. However, several methods and apparatuses for cancer treatment with hyperthermia, (such as prostate or breast cancer, or any other soft tissue cancerous or benign mass) have been invented and are currently available. For example, in [3] an invention was presented that relates to a method for treating a cancer including a combination of a treatment by a nucleic acid molecule mimicking double strand breaks with hyperthermia. In [4] the investigators employed a three-dimensional software-controlled electronic amplifier array using arbitrary waveforms that dynamically and proportionally steer electrical currents by using two or more current vector paths, sequentially or simultaneously, through a mass containing electrically-conductive ionic solutions so as to obtain 100% thermal heating or hyperthermia through the mass, and destroying it with a minimally-invasive treatment that requires no radiation or chemotherapy, which could be harmful to the patient. Another invention [5] provides an immunomodulator for use in the treatment and/or control of a neoplastic disease in a patient intended to undergo immunogenic cell death therapy simultaneously, separately or sequentially with administration of the immunomodulator. The therapy can be selected from microwave irradiation, targeted radiotherapy, embolization, cryotherapy, ultrasound, high intensity focused ultrasound, cyberknife, radiofrequency ablation, cryoablation, electrotome heating, hot water injection, alcohol injection, radiation exposure, photodynamic therapy, laser beam

irradiation, but also hyperthermia and combinations thereof. A very interesting method of thermo-acoustic tomography and hyperthermia was introduced in [6], and in [7] certain methods and instrumentation for ultrasound mediated delivery of drugs to diseased tissue were presented. These devices use ultrasound beams with frequency and focusing that provides an ultrasound radiation force acting on the drug and surrounding fluid that produces a convection of drugs and compensates for the lack of a pressure gradient. To manipulate drug encapsulations and also stimulate transport of drugs across biological membranes, like the cell membrane or the blood brain barrier, devices to use low frequency beams with high mechanical index. Along with these devices, additional ultrasound heating of the tissue is used to increase blood flow and manipulate thermally sensitive particles. With respect to ultrasonic energy applications, one finds in [8] a compact, high-power, dual-mode, emitting and receiving ultrasound transducer and a method for applying ultrasonic energy within a living subject and for monitoring the effects it induces in tissue. The device invented in [8] comprises a set of piezoelectric polymeric transducer elements and a set of piezoelectric ceramic elements, bonded together. The polymeric transducer elements have electrodes enabling their use for low-power diagnostic imaging interrogation of the tissue and the ceramic transducer elements have electrodes enabling their use for high-power therapy applications. Furthermore, a method and composition for hyperthermally diagnosing and monitoring treatment of cells in an animal with photoacoustic sound and nanoparticles was introduced in [9]. The heat (temperature) and photoacoustic sound-wave production inside the target tissue is measured. The desired temperature is achieved using a laser and photoacoustic imaging technique. Hyperthermia treatment of tissue in a target site applies a heat source to kill cells without protein denaturation. The hyperthermia treatment may further comprise platelet-derived treatment. The method introduces an encapsulated dye that is released at a selected temperature in the target site to indicate that a threshold temperature has been reached to hyperthermally treat the tissue. In one embodiment, the composition releases the dye at a temperature of 42°C to 56°C, but preferably between 45°C to 49°C. The composition, which can be a liposome composition encapsulating the dye, can be introduced to the bloodstream of the patient to flow through the target site. Another sophisticated invention [10] relates to an array of radiative antenna elements for combined MR

CHAPTER 8

Circulating and Local Estrogen Concentrations are Protective against Breast Cancer in Obese Women

Zsuzsanna Suba*

National Institute of Oncology Budapest, Budapest, Hungary

Abstract: Literary data suggest apparently ambiguous interaction between menopausal status and obesity-associated breast cancer risk based on the principle of the carcinogenic capacity of estrogen. Before menopause, breast cancer incidence is relatively low and adiposity is erroneously regarded as a protective factor against this tumor conferred by the obesity associated defective estrogen-synthesis. By contrast, in postmenopausal cases, obesity presents a strong risk factor for breast cancer being mistakenly attributed to the presumed excessive estrogen-production of their adipose-tissue mass. Obesity is associated with dysmetabolism and endangers the healthy equilibrium of sexual hormone-production and regular menstrual cycles in women, which are the prerequisites not only for reproductive capacity but also for somatic health. At the same time, literary data support that anovulatory infertility is a very strong risk for breast cancer in young women either with or without obesity. In the majority of premenopausal women, obesity associated insulin resistance is moderate and may be counteracted by their preserved circulatory estrogen level. Consequently, it is not obesity but rather the still sufficient estrogen-level, which may be protective against breast cancer in young adult females. In obese older women never using hormone replacement therapy (HRT), the breast cancer risk is high, which is associated with their continuous estrogen loss and increasing insulin-resistance. By contrast, obese postmenopausal women using HRT, have a decreased risk for breast cancer as the protective effect of estrogen-substitution may counteract to their obesity associated systemic alterations. Increased local estrogen synthesis in the microenvironment of breast malignancies may be regarded as defensive counteraction against tumor spread. The revealed inverse correlation between estrogen-levels and breast cancer risk in obese women should advance our understanding of breast cancer etiology and promotes primary prevention measures. New patents recommend various methods for the prevention and treatment of obesity-related systemic disorders and the associated breast cancer.

* **Corresponding author Dr. Zsuzsanna Suba:** National Institute of Oncology Surgical and Molecular Tumor Pathology Centre Address: H-1122 Ráth György str. 7-9 Budapest, Hungary; Tel: 00 36 1 224 86 00; Fax: 0036 1 224 86 20; E-mail: subazdr@gmail.com

Atta-ur-Rahman and Khurshid Zaman (Eds)

Keywords: Androgen, antiestrogen, breast cancer risk, estrogen, insulin resistance, menopause, metabolic syndrome, obesity, type-2 diabetes, visceral adiposity.

INTRODUCTION

Many studies support an apparently Janus-faced ambiguous interaction between obesity and breast cancer risk depending on the menopausal status of patients [1 - 3]. In young women, before menopause adiposity is erroneously regarded to exhibit a protective effect against breast cancer risk [1, 4, 5]. By contrast, in postmenopausal cases, particularly in the elderly, the association is highly positive, obesity confers a strong risk for breast cancer [2, 6]. Controversial results of clinical studies mistakenly suggest that obesity is advantageous against breast cancer by the defective estrogen synthesis in young women [1]. This assumption seems to be consistent with an inverse relationship between the BMI and serum estradiol levels found in premenopausal cases, particularly in the follicular phase of the cycle [7]. Conversely, postmenopausal obesity is harmful by the erroneously presumed excessive estrogen production of adipose tissue mass in older women [8 - 10]. Explanations for these supposed ambiguous correlations are in concordance with the preconception that high estrogen levels, either endogenous or exogenous, play crucial role in mammary carcinogenesis [11].

Nevertheless, several authors could find confusing and disturbing associations between obesity and breast cancer risk in postmenopausal women. Obese postmenopausal women, who had never used hormone replacement therapy (HRT) exhibited fairly high breast cancer risk [12]. By contrast, HRT use attenuates or abolishes the increased breast cancer incidence [13 - 15], suggesting a protective impact of female sexual hormones in aged obese women.

In premenopausal cases, the results of clinical studies justify that obesity induces mild or moderate decrease in circulating estrogen levels reflected by their inclination to anovulatory infertility and long or irregular menstrual cycles [16]. Based on the traditional concept of estrogen induced mammary carcinogenesis, obesity associated defective estrogen production in premenopausal women seems apparently to justify the breast cancer preventive impact of fatness.

Even larger studies, which equivocally strengthen the protective effect of obesity for premenopausal breast cancer risk, confess that the responsible mechanisms are completely obscure. Evidences, provided by clinical endocrinological studies regarding correlations between defective hormonal status of obese women and decreased breast cancer incidence, are inconsistent or fairly contradictory [1]. Considering the whole spectrum of unexplained, apparently ambiguous biological behavior as regards breast cancers arising in premenopausal and postmenopausal women, the existence of two distinct types of breast malignancies was presumed occurring before and after menopause [17, 18].

Obesity is a well-known cancer risk factor associated with different grades of insulin resistance and a disturbance of male to female circulating sexual steroid levels [19, 20]. Hyperinsulinemia, dyslipidemia, elevated fasting glucose level and type-2 diabetes are the well documented concomitants of adiposity associated insulin resistance [21]. Obesity provokes further alterations in the endocrine system as well conferred by an excessive circulatory androgen level at the expense of defective estrogen synthesis [22]. Nevertheless, the health benefit of pathologic states; such as overweight and obesity can hardly be justified at any age of women.

The aim of the present study is to clarify the realistic correlations between breast cancer risk and obesity-associated hormonal alterations during the whole life of women. More-over, this study tries to reveal the sources of the misleading clinical and epidemiologic results suggesting the breast cancer protective effect of obesity in the young. These asso-ciations are examined in an analytical review based on the results of prospective, case-control and meta-analytic studies.

PATHOGENETIC MECHANISMS AS LINKS BETWEEN OBESITY AND BREAST CANCER RISK

Clinical and experimental evidences prove that obesity, particularly visceral fatty tissue deposition leads to insulin resistance,associated with diverse immunologic, metabolic and hormonal alterations mediating breast cancer risk (Fig. (**1**)). The main stream of obesity related alterations is a self-generating, progressive insulin resistance in thorough interplay with the dysmetabolism and inflammation of

CHAPTER 9

Recent Patents on Polymeric Nanosystems Applications for Anticancer Drug Delivery

María E. Ruiz[1], Melisa E. Gantner[2], Alan Talevi[3,*]

[1] Quality Control of Medications, Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP) - Argentinean National Council for Scientific and Technical Research (CONICET) CCT La Plata. 47 and 115, La Plata (B1900AJI), Buenos Aires, Argentina

[2] Medicinal Chemistry, Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP) - 47 and 115, La Plata (B1900AJI), Buenos Aires, Argentina

[3] Biopharmacy, Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP) - Argentinean National Council for Scientific and Technical Research (CONICET) CCT La Plata. 47 and 115, La Plata (B1900AJI), Buenos Aires, Argentina

Abstract: Nano-sized drug delivery systems might well be a fundamental tool to overcome some of the major challenges for future treatment of cancer, optimizing drug effectiveness while simultaneously reducing systemic side effects. This chapter is an update on patents related to nanosystems applications to anticancer drug delivery published in the last five years (2010-2015); the chapter focuses on polymeric nanosystems (nanospheres, nanocapsules, hydrogel based nanosystems, dendrimers and polymeric micelles).

Keywords: Anticancer drugs, biopharmaceutics, cancer therapy, drug delivery, dendrimers, hydrogels, multifunctional systems, nanocapsules, nanomedicine, nanoshells, nanospheres, nanosystems, nanotechnology, patents, pharmacokinetics, polymeric micelles, stimuli-responsive systems, cancer, tumors, hybrid nanosystems, polymer-based nanocarriers, polymeric nanocarriers.

[*] **Address Correspondence to this Author Alan Talevi:** Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP) - Argentinean National Council for Scientific and Technical Research (CONICET) CCT La Plata. 47 and 115, La Plata (B1900AJI), Buenos Aires, Argentina. Tel: 542214235333; Ext 41; E-mail: atalevi@biol.unlp.edu.ar, alantalevi@gmail.com

Atta-ur-Rahman and Khurshid Zaman (Eds)

1. INTRODUCTION

Despite the development of novel anticancer drugs is a burgeoning research field that has provided promising results in the last decade [1, 2], the biopharmaceutical, pharmacokinetical and safety aspects of current and incoming anticancer drug therapies are particularly critical to achieve the desired clinical outcome in the field of oncology. Thus, the discovery of novel chemotherapies should be complemented with the design of innovative delivery systems. As a matter of fact, several efficacy and safety issues of presently used chemotherapeutic agents are related to pharmacokinetic issues, e.g. fast clearance or unspecific biodistribution. For instance, Vinca alkaloids, anthracyclines, epipodophyllotoxins, Taxol and Actinomycin D are substrates of ATP-binding Cassette (ABC) transporters linked to multi-drug resistance issues [3 - 5], while other such as 5-Fluorouracil, Camptothecin, Gemcitabine and Curcumin are very rapidly metabolized or inactivated in the physiological environment [6 - 9].

Nanoparticulated systems present great promise as delivery vectors for anticancer therapeutics: they can be uptaken by the cells through specialized uptake mechanisms; they can improve the stability of pharmaceutical active ingredients in physiological conditions and they are biocompatible when synthesized from biodegradable materials [10]. What is more, they can be designed for either passive, active or smart targeting of cancerous tissues [11 - 16].

Particularly, polymer-based pharmaceutical nanocarriers display numerous advantages in contrast with other compositions: they are ideal candidates for targeted delivery owing to their tunable properties and the possibility to design systems sensitive to a number of external stimuli; they are stable and allow high loading of diverse therapeutic agents; they provide control over drug release kinetics and; many polymers have a long history of safe use in humans [17, 18]. This chapter focuses on patents related to polymer-based organic nanosystems (nanospheres, nanocapsules, nanogels, dendrimers and nanomicelles) published in the last five years. We have omitted those inventions related to general drug delivery systems which are not specifically directed to cancer treatment, or which do not present examples/embodiments related to cancer therapy.

1.1. Nanoparticles as Drug Carriers: General Considerations

Nanoparticles (NP) can accumulate in tumors cells by diffusing through cancer leaky blood vessels followed by uptake to cancer cells via endocytosis [19 - 25]. The pore cutoff size of cancer blood capillaries has been reported in the range from 380 to 780 nm [21, 26, 27].

This leaky nature allows a relatively easy extravasation of large molecules or colloids to the cancerous tissues. In addition, tumor tissues frequently lack effective lymphatic drainage. Jointly, these two features explain the Enhanced Permeability and Retention (EPR) effect [23, 28]. NP have thereof been used to passively control the distribution of anticancer drugs to normal cells and tissues, limiting off-target effects [29], providing physical and chemical protection from rapid blood clearance [30] and solving low aqueous solubility issues [29].

Due to their small size, NP may be administrated through different routes such as intravenous (IV), subcutaneous (SC), etc. Such small size can be exploited to increase solubilization and drug release.

The clearance kinetics and biodistribution of nanosystems depend on physicochemical and biochemical factors such as particle size, composition, surface charge, hydrophobicity and surface decoration, all of which can be tailored for targeting purposes [31, 32]. Once in systemic circulation and after opsonization by blood proteins, drug delivery nanosystems tend to be rapidly removed by the mononuclear phagocyte system (MPS) [33].

While the natural tendency of nanosystems to localize in the MPS represents an excellent opportunity to target drugs to the macrophages present in the liver and the spleen [34, 35], it becomes a major obstacle to deliver drugs whose site of action is located in other organs.

A variety of methods have been explored to prevent opsonization and the subsequent MPS uptake, prolonging circulation time of the NP [36 - 39]. Steric stabilization of NP is achieved by adsorbing hydrophilic surfactants or block/branched copolymers on their surface (stealth coating), PEGylation (PEG: polyethylene glycol) being the most frequent strategy [31, 40, 41] in spite of some

CHAPTER 10

Recent Patents on Nanosystems Applications to Anticancer Drug Therapy: Lipid-based Systems

Alan Talevi[1,*], María E Ruiz[2]

[1] *Biopharmacy, Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP) - Argentinean National Council for Scientific and Technical Research (CONICET) CCT La Plata. 47 and 115, La Plata (B1900AJI), Buenos Aires, Argentina*

[2] *Quality Control of Medications, Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP) - Argentinean National Council for Scientific and Technical Research (CONICET) CCT La Plata. 47 and 115, La Plata (B1900AJI), Buenos Aires, Argentina*

Abstract: The advent of nanomedicines allows a diversity of drugs with unfavorable biopharmaceutical properties and safety issues to be accessible as pharmaceutical products. The expectations are particularly high in the field of cancer therapeutics, where nanotechnology promises to enhance treatment selectivity, solving or ameliorating off-target toxicity issues. The biocompatible nature of lipid-based nanosystems and their scalable production make them especially auspicious for therapeutic applications.

Here, we present an update on recent patents on lipid-based nanosystems (liposomes, solid lipid nanoparticles and nano-structured lipid carriers) applications to cancer therapy.

Keywords: Anticancer drugs, biopharmaceutics, cancer, cancer therapy, lipid-based nanosystems, liposomes, multifunctional nanosystems, nanomedicine, nanostructured lipid carriers, nanosystems, nanotechnology, patents, pharmacokinetics, pharmaceutical nanocarriers, smart delivery, solid lipid nanocarriers, stimuli-responsive systems, targeted drug delivery.

[*] **Corresponding author Alan Talevi:** Biopharmacy, Department of Biological Sciences, Faculty of Exact Sciences, National University of La Plata (UNLP) - Argentinean National Council for Scientific and Technical Research (CONICET) CCT La Plata. 47 and 115, La Plata (B1900AJI), Buenos Aires, Argentina. Tel: 542214235333; Ext 41; E-mail: atalevi@biol.unlp.edu.ar, alantalevi@gmail.com

Atta-ur-Rahman and Khurshid Zaman (Eds)

1. INTRODUCTION

According to the 2015 report of the American Cancer Society (ACS) [1], cancer is the second most common cause of death in the US, exceeded only by heart disease, and accounts for nearly 1 of every 4 deaths. About 1,658,370 new cancer cases are expected to be diagnosed in 2015, and, only in the US, about 1,620 people per day are expected to die of cancer in 2015.

As a positive aspect of this worrying scenario, the earlier diagnosis of certain cancers and advances in treatment have improved the survival rates: the 5-year relative survival rate has slowly increased, from 49% for all cancers diagnosed in 1975-1977 up to 68% in 2004-2010. Despite the fact that these survival rates do not reflect the most recent advances in detection and treatment (since they are based on patients who were diagnosed from 2004 to 2010), the ACS report is a sad reminder of the importance of anticancer research.

It is critical to address the matter in a multi-objective manner, where different approaches may contribute to improve outcomes for patients affected by the disease. As stated by the National Cancer Institute [2], these approaches may include the development of more effective and less toxic treatments (such as targeted therapies, immunotherapies, and cancer vaccines); the improvement of already known therapies (chemotherapy, radiation therapy, surgery); and the improvement of a patient's ability to receive effective cancer treatment by a better management of the treatment's toxic effects, among others.

Last generation pharmaceutical nanocarriers are an interesting option to reduce the side effects associated with the current anticancer drugs. Conventional drug delivery systems rely on establishing a dynamic equilibrium between the free drug plasmatic concentration and the free drug concentrations in extravascular tissues, including the targeted tissue. Since only the free, unbound drug can interact with its molecular target, the free drug levels at the vicinity of the site of action will determine the extent of the pharmacological response [3]. A non-trivial implication of the former approach is that, to reach effective levels of a pharmaceutical active ingredient in its site of action, the patient is subjected to systemic exposure to the drug, which often leads to off-target side effects. In other

words, conventional drug delivery systems are characterized by non-targeted (non-specific) distribution. Patients receiving anticancer treatment constitute a very illustrative example of the consequences of the previous setting: the well-recognized adverse reactions to chemotherapy majorly emerge from interactions between the drug molecules and non-cancerous, healthy cells. These side-effects could then be ameliorated or avoided if targeted drug delivery systems were used.

On the other hand, a number of active ingredients cannot be fully exploited due to biopharmaceutical/pharmacokinetic issues. For example, a given drug might be non-compatible with certain routes of administration: frequently, drugs with low aqueous solubility cannot be formulated as IV solution. Drugs with poor gastrointestinal absorption, high first pass metabolism or low chemical stability in the gastrointestinal media often compromise oral administration. Active ingredients with short half-life present difficulties to build up and sustain effective levels (reducing the duration of the pharmacological effect or requiring large doses just to compensate biotransformation). Finally, interaction of the free drug with efflux transporters from the ABC superfamily (e.g. Multi-Drug Resistance Proteins) results in a reduced bioavailability and is associated to multi-drug resistance phenomena in cancer [4 - 6].

Some of the previous difficulties are very frequent in the case of emerging therapies, particularly, biotherapies (gene therapy, therapeutic proteins): the intrinsic nature of complex macromolecules makes them more susceptible to enzymatic cleavage, inactivation, poor permeability, slow distribution and immunogenicity [7 - 11]. As a result, the development of adequate delivery vectors is a key issue in the field of next generation therapeutics. Advanced delivery systems for anticancer drugs should be able to retain their integrity throughout the drug absorption and distribution events, selectively releasing their cargo in the proximity of the drug target. An ideal drug delivery system should, thus: a) compensate unfavorable physicochemical properties of the active ingredient; b) encapsulate, entrap, adsorb or conjugate drug molecules; c) conceal the drug from enzymatic cleavage, excessively rapid biotransformation and recognition by efflux transporters; d) direct the drug to its molecular target; in the case of intracellular targets, promote cell uptake; e) release the drug load in a controlled manner (in the vicinity of the molecular target and not before, at a

CHAPTER 11

Novel Patents and Cancer Therapies for Transforming Growth Factor-β and Urokinase Type Plasminogen Activator: Potential Use of Their Interplay in Tumorigenesis

Jelena Krstić[1], Irina Maslovarić[2], Hristina Obradović[1], Tamara Kukolj[1], Juan F. Santibanez[1,*]

[1] *Laboratory for Experimental Hematology and Stem cells. Institute for Medical Research, University of Belgrade*

[2] *Laboratory for immunochemistry. Institute for Medical Research, University of Belgrade*

Abstract: Transforming growth factor beta1 (TGF-β1) plays different roles in health and disease. TGF-β1 has been assumed as a dual factor in tumor growth, since it can repress epithelial tumor development in early stages, while it acts as a tumor promoter in the late stages of tumor progression. Cancer cells, during carcinogenesis, acquire migration and invasion capacity which enables them to metastasize. The urokinase type plasminogen activator (uPA) system, comprised of uPA, the cell surface receptor (uPAR) and plasminogen-plasmin, is involved in the proteolytic degradation of the extracellular matrix and it also regulates several critical cellular events by its capacity to trigger the activation of intracellular signaling pathways. This enables the cancer cell survival, its dissemination, and enhancement of cell malignancy during tumor progression. The expression of both uPA and uPAR is finely regulated in normal development, but their expression is deregulated in cancer. TGF-β regulates uPA expression in cancer cells while uPA, by conversion of plasminogen to active form, plasmin, may release TGF-β1 from its latent state. Thus, these pathways cross-regulate each other by mutual feedback contributing to tumor progression. Here, we review the specific roles and the interplay between TGF-β1 and uPA system in cancer cells, the current cancer therapies and the novel patents focused mainly on uPA and TGF-β ligands and their cell surface receptors. Finally, with regard to the mutual activity of uPA and TGF-β1 in tumorigenesis, the aim of this chapter is to expose the potential of TGF-β1 and uPA systems to become combinatorial targets for therapies and patents.

[*] **Address Correspondence to this Author:** Laboratory for Experimental Hematology and Stem cells. Institute for Medical Research, University of Belgrade. Dr Subotića 4, 11129 Belgrade, Serbia Tel: +381 11 2685 788, Fax: +381 11 2643 691, E-mail: jfsantibanez@imi.bg.ac.rs

Atta-ur-Rahman and Khurshid Zaman (Eds)

Keywords: Activin A receptor type II-like kinase 5, ALK5, antisense oligonucleotides, anti-TGF-β antibodies, cancer, clinical trials, ECM, endoglin, extracellular matrix, fusion protein, microRNA, oligodeoxynucleotide, PAI, patents, plasminogen activator inhibitor, plasminogen-plasmin, soluble-uPAR, suPAR, TGF-β, therapy, transforming growth factor-β, transforming growth Factor-β receptor, Type I TβRI, Type II Transforming growth Factor-β receptor, TβRII, type III non-kinase TGF-β receptor, uPA, uPAR, urokinase type plasminogen activator, urokinase type plasminogen activator receptor.

INTRODUCTION

Metastasis results from a complex molecular cascade, which allows cancer cells to leave the primary site and disseminate to distant anatomic sites where they proliferate and form secondary tumor foci. Disseminated disease is the most usual cause of death in cancer patients, and it is therefore a very serious clinical problem [1].

Transforming growth Factor-β 1 (TGF-β1) has been implicated in tumor progression as a dual factor, acting as a tumor suppressor in early stages of carcinogenesis, and exerting a pro-oncogenic role in the last steps of the metastatic disease [2]. TGF-β1 induces the epithelial mesenchymal transition (EMT) of transformed cells, which contributes to tumor invasion and metastasis, and is frequently over-expressed in carcinoma cells [3 - 7].

To invade and metastasize, cancer cells traverse the surrounding extracellular matrix (ECM) expressing a set of ECM degrading proteases, such as urokinase type plasminogen activator (uPA), which plays a key role in cell invasion and metastasis. uPA converts plasminogen to plasmin, which in turn can degrade a wide variety of ECM components, and enables the tumor cells to penetrate the basement membrane [8, 9]. In addition, uPA, by binding to its cell surface receptor (uPAR), also modulates cell adhesion, proliferation and migration [10, 11]. Consistent with its role in cancer dissemination, high level of uPA correlates with adverse patient outcome [9, 12].

The aim of this chapter is to reveal the key interplay between TGF-β1 and uPA systems in tumorigenesis, and the therapies and recent patents addressing either

TGF-β1 or uPA in cancer. Considering that both systems are followed by a complex cascade of events that culminates in an enhanced malignancy of tumor cells, this could open up an opportunity for future development of new patents by simultaneous addressing of both TGF-β1 and uPA systems, contributing this way to the development of new cancer therapies in compliance with current medical protocols.

TRANSFORMING GROWTH FACTOR BETA

Transforming growth factor-β1 belongs to a large family of structurally related regulatory proteins that comprises more than 40 proteins expressed in mammals, including activins, inhibins, bone morphogenetic proteins and growth and differentiation factors, among others [13]. TGF-β1 has been involved in a plethora of different biological processes, which include cell growth, differentiation and development, as well as tumorigenesis [14]. Among the TGF-βs, mammals express three genetically distinct isoforms (TGF-β1, -2 and -3) with high homology. The corresponding human genes are located on chromosomes 19q13, 1q41 and 14q24, respectively [13, 14].

TGF-βs initiate signaling, (Fig. (**1**)) by binding to its cell-surface serine/threonine kinase receptors type I and II (TβRI also named activin A receptor type II-like kinase 5 (ALK5) and TβRII), which form heteromeric complexes in the presence of dimerized ligands. Binding of TGF-β to TβRII leads to the phosphorylation and activation of TβRI/ALK5 [15]. The cytoplasmatic mediators Smad2,3 are phosphorylated by the activated receptors, further inducing the release of Smads from the complex formed with Smad anchor for receptor activation (SARA) from the inner side of plasma membrane. Activated Smad2,3 afterwards form a complex with the common mediator Smad4 to be translocated into the nucleus, where it regulates the expression of different target genes by interacting with several transcription factors, co-activators or repressors [16]. In endothelial cells (EC), TGF-β1 may signal through two different type I receptors, ALK1 and ALK5 [17, 18]. Activation of ALK1 by TGF-β1 induces the activation of Smad 1, 5 and 8, stimulating EC proliferation, migration and tubulogenesis [19], and is associated with the activation phase of angiogenesis.

CHAPTER 12

Novel Compounds and Drugs and Related Patents in Lung Cancer Chemotherapy

Attapon Cheepsattayakorn[1,*], Ruangrong Cheepsattayakorn[2]

[1] *10th Zonal Tuberculosis and Chest Disease Center, 143 Sridornchai Road Changklan Muang Chiang Mai 50100 Thailand and 10th Office of Disease Prevention and Control, Department of Disease Control, Ministry of Public Health, Thailand*

[2] *Department of Pathology, Faculty of Medicine, Chiang Mai University, Chiang Mai, Thailand*

Abstract: Lung cancer affecting the majority of patients is usually present with advanced stage and contributes in killing more people than any other malignancy in the world. The discovery of a number of lung cancer-molecular alterations contributes to uniquely targeted therapies with specific inhibitors for non-small cell lung cancer such as erlotinib, gefitinib and crizotinib.

Pemetrexed has statistically shown significantly reduced adverse-side effects of drug compared with docetaxel. V1801, an analog of gefitinib may overcome gefitinib resistance in patients with non-small cell lung cancer. Thymosin α_1, an immunomodulator, significantly improves patient's quality of life by enhancing T-cell function, stimulation of T-cell maturation and differentiation. Various novel compounds and chemotherapeutics were introduced in 2013 patents such as taxane, quinazoline, arylamino purine, benzodiazepine, pyrrolopyrimidine, nitrobenza-mide, cyclopropane amide, 4-iodo-3-nitrobenzamide, heteroaryl (alkyl) dithiocarba-mate, and histone deacetylase in treating non-small-cell lung cancer and piperidine, piperazine, picoplatin, and arsenic trioxide in treating small-cell lung cancer. Currently, new drugs such as alectinib, AP26113, crizotinib, etc. are in the pipeline.

Keywords: ALK, BRACA1, cancer, carboplatin, chemotherapy, compounds, docetaxel, drugs, EGFR-TKI, ERCC1, erotinib, FANCD2, KRAS, lung, pemetrexed, platinum-based, RAD51, related patents, SMO, VEGF.

[*] **Address correspondence to this author:** 10th Zonal Tuberculosis and Chest Disease Center, 143 Sridornchai Road Changklan Muang Chiang Mai 50100 Thailand and 10th Office of Disease Prevention and Control, 447 Lumphun Road Watgate Muang Chiang Mai 5000, Thailand. Tel: 66 89 6344075, 66 53 140767, 66 53 276364; Fax: 66 53 140773, 66 53 273590; E-mail: Attapon1958@gmail.com

Atta-ur-Rahman and Khurshid Zaman (Eds)

INTRODUCTION

The aim of this study is to review the literature and related patents which demonstrated the progress of lung cancer chemotherapy. There is a previous study that demonstrated the correlation between increased microsatellite alterations and HIV-related lung cancer due to genomic instability [1]. Tobacco is the leading cause of preventable deaths around the world [2]. Half of the regular smokers die prematurely of tobacco-related diseases [3]. Tobacco elimination is the single most effective method available to address the dismal statistics associated with lung cancer [4]. Lung cancer is affecting the majority of patients, usually present with advanced stage and contributes in killing more people than any other malignancy in the world [5]. Nanoscale devices have potential to change the lung cancer chemotherapy by customizable, targeted drug delivery vehicles capable of ferrying larger doses of therapeutic genes or agents into the malignant cells with sparing healthy cells and reduce many cancer-chemotherapeutic side effects [6]. The discovery of a number of lung cancer-molecular alterations contribute to uniquely targeted therapies with specific inhibitor drugs such as crizotinib for gene translocation resulting in the echinoderm microtubule-associated protein-like 4 (*EML4*)-anaplastic lymphoma kinase (*ALK*) oncogene or erlotinib and gefitinib for mutations in the epidermal growth factor receptor (EGFR) [5]. Use of liposomes (or small sphere) is the earliest well-known nanotechnological application which has been used to treat cancers for over 15 years [7]. Torchilin *et al.* demonstrated that formulated antitumor antibody-conjugated polymeric micelles (immunomicelles), encapsulating the water-insoluble drug, paclitaxel effectively bind to various cancer cells including human lung cancer cells *in vitro* [8, 9]. Rawat *et al.* [10] and Choi *et al.* [11] studied that dendrimer-5-fluorouracil conjugates were prepared by acetylation which released free 5-fluorouracil upon hydrolysis, minimizing the 5-fluorouracil toxicity. A previous study by Yin *et al.* revealed tumor growth inhibition by endohedral metallofullerenol nanoparticles, a type of fullerenes optimized as reactive oxygen species scavengers and demonstrated that $Gd@C_{82}(OH)_{22n}$ nanoparticles reduced H_2O_2-induced reactive oxygen species formation and mitochondrial damage with efficient inhibition of the growth of malignant tumor *in vivo* (Gd = Galldolinium) [12]. Liposomes, artificially prepared vesicles composed of a lipid bilayer that can

be filled with drugs and delivered drugs for cancers are currently being used for anticancer drug delivery such as ONCO-TCS® [13], DepoCyt® [14, 15], DaunoXome® [16, 17], Doxil® [18, 19]. Major problems associated with liposomal nanoparticles are difficulty in sterilization, low drug loading capacity, batch to batch reproducibility, and their stability [6].

DESIGNING ANTI-CANCER DRUGS

Over the last 50 years, only about 25 natural and synthetic chemical compounds of about 500,000 natural and synthetic compounds have been tested for anti-cancer activity. Fifty genes of 100,000 genes in a human cell are known as proto-oncogenes. If 5 or 6 of these proto-oncogenes accumulate critical mutations in a cell, the subtle changes are likely to result in a fully malignant cell and have potential formation of a tumor. The drugs used to fight against cancer are classified into 2 broad categories. The first is cytostatic (cell- stabilizing activity) and the second is cytotoxic (cell-killing activity). Both categorized drugs contribute to a reduction of the tumor size by prevention of the tumor cell population division [20]. Costa *et al.* developed and validated a polymerase chain reaction (PCR)-based assay for the detection of the EGFR mutations in plasma or serum samples by offering higher analytical sensitivity by enhancing amplification of mutant alleles in the samples using a protein-nucleic-acid probe compared to standard methods, in which primers flanking the mutations are employed for PCR amplification and further sequencing analysis [21].

CHEMOTHERAPY FOR NON-SMALL-CELL LUNG CANCER (NSCLC : SQUAMOUS CELL OR EPIDERMOID CARCINOMA, ADENOCARCINOMA, LARGE CELL CARCINOMA, AND UNDIFFERENTIATED CARCINOMA)

Morrison *et al.* presented the methods of classifying biological specimens for predicting response to TKI treatment in NSCLC patients with poor outcomes on second- or third-line gefitinib therapy [22]. Wang *et al.* introduced in the US Patent Application Number: US20130225811 quinazoline derivatives and quinazoline complexes which showed good inhibitory effect on proliferation of various tumor cells including human NSCLC cell line A549 [23]. TOR kinase

CHAPTER 13

The Rationale of Immunogenic and Effective Naked DNA Vaccines Against Cancer: Latest Advances

Daniela Fioretti[≠], Sandra Iurescia[≠], Monica Rinaldi[*]

Institute of Translational Pharmacology (IFT), National Research Council (CNR), Via Fosso del Cavaliere 100 - 00133 Rome, Italy

Abstract: A variety of clinical trials for vaccines against cancer have provided evidence that DNA vaccines are well tolerated and have an excellent safety profile.

DNA vaccines require much improvement to make them sufficiently effective against cancer in the clinic. Nowadays, it is clear that an increased antigen expression correlates with improved immunogenicity and it is critical to vaccine performance in large animals and humans. Similarly, additional strategies are required to activate effective immunity against poorly immunogenic tumor antigens.

This chapter discusses very recent scientific references focused on the development of sophisticated DNA vaccines against cancer.

We report a selection of novel and relevant patented inventions employed to improve DNA vaccine immunogenicity through several strategies such as the use of tissue-specific transcriptional elements, nuclear localisation signalling, codon-optimisation and by targeting antigenic proteins to secretory pathway.

Recent patents validating portions or splice variants of tumor antigens as candidates for cancer DNA vaccines with improved specificity, such as mesothelin and hTERT, are also discussed.

Lastly, we review novel scientific references and patents on the use of genetic immunomodulators, such as "universal" T helper epitopes derived from tetanus toxin, *Escherichia coli* heat labile enterotoxin and vegetable proteins, as well as cytokines, c-

[*] **Address correspondence to this author:** Institute of Translational Pharmacology, National Research Council, *Via* Fosso del Cavaliere 100 - 00133 Rome, Italy; Tel: +39 0649934219; Fax: +39 0649934257; E-mail: monica.rinaldi@ift.cnr.it

[≠] These authors contributed equally to this work

Atta-ur-Rahman and Khurshid Zaman (Eds)

hemokines or costimulatory molecules such as IL-6, IL-15, IL-21 to amplify immunity against cancer.

Keywords: Antigen targeting, cancer, clinical trials, chemokine, codon optimisation, codon usage, costimulatory molecule, cytokine, DNA vaccine, epitope optimisation, epitope selection, genetic adjuvant, immunogenicity, immunotherapy, intracellular compartment addressing, nuclear localisation signalling, plasmid backbone, plasmid delivery, T cell help, tumor antigen.

INTRODUCTION

The process of shaping tumor immunogenicity has been termed cancer immunoediting and the composite result of the process determines the outcome of tumor rejection, dormancy, or progression. Compatible with the cancer immunoediting hypothesis, the presence of clinically apparent tumors indicates a failed attempt to control tumor progression by the host immunity due to its ineffectiveness or acquired tolerance [1, 2]. Thus, the clinical goal of cancer immunotherapy is to elicit an effective anti-tumor immunity by engendering productive immune responses and breaking tumor-induced immune tolerance.

Cancer immunotherapy is fast gaining global attention with its unique position as a potential therapy showing promise in cancer prevention and cure. Immunotherapy offers a unique strategy in cancer therapeutics because it utilises the natural system of immunity, and it is therefore less toxic compared to conventional chemotherapy and radiotherapy that also destroy healthy haematopoietic, endothelial and stromal cells.

The aim of tumor immunotherapy is to provide either passive or active immunity against malignancies by harnessing the immune system to target tumors [3]. Active immuno-therapy uses the host's immune system that can discriminate cancer cells from normal cells based on tumor antigen recognition [4]. Although some active immunotherapies are designed to induce antibodies as the primary effector mechanism, induction of antigen-specific T cell responses is the primary objective of active immunotherapy [5]. Approaches that directly incorporate tumor antigen are conventionally referred to as vaccines. Whereas immune modulators and antibodies represent a passive form of therapy, cancer vaccines

require a functional immune system to be active [5]. In this context, cancer vaccines may serve as ideal treatment due to their specificity for tumor cells and long lasting immunological memory that may safeguard against recurrences [6]. Cancer vaccines including tumor cell- and tumor antigen-based vaccines are all examples of active immunotherapy [7, 8].

Significant advances have been made in the use and development of cancer vaccines. According to their specific formulation, cancer vaccines can be divided into various categories: 1) subunit vaccines, 2) autologous cell vaccines, 3) allogeneic cell vaccines, 4) DNA-based vaccines, 5) dendritic cell-based vaccines. Often, most of these vaccine formulations include adjuvants, which serve to potentiate the innate immune response (reviewed in [9]).

Among these approaches, DNA-based vaccines have considerable promise. The effectiveness to screen for antigens rapidly and to design specific types of expression constructs has made the study of DNA vaccines a valuable field for antigen-specific immunotherapy of cancer.

Induction of effective immune attack on cancer cells in patients requires conversion of weak tumor antigens into strong immunogens. A recent study described a DNA vac-cination strategy able to overcome immune tolerance to self-tumor antigen and reported successful therapeutic outcomes in a preclinical model of metastatic melanoma [10].

Furthermore, the active process of ‘tolerisation’ taking place in the tumor micro-environment (TME) must be overcome if the goal is to produce active immunity. In a recent study on breast cancer, the use of DNA vaccines directed against components of TME, as tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs) overexpressing specific targets for therapy, resulted in the elimination of tumor growth, progression, metastasis and recurrence in mouse tumor models [11].

DNA vaccines allow incorporating multiple components to activate and direct selected immune effector pathways [12]. The DNA platform is conceptually safer and more stable than are conventional vaccine approaches. The original concerns associated with the DNA platform were the potential for genomic integration and

Author Index

Atta-ur-Rahman and Khurshid Zaman (Eds)

SUBJECT INDEX

A

B

C