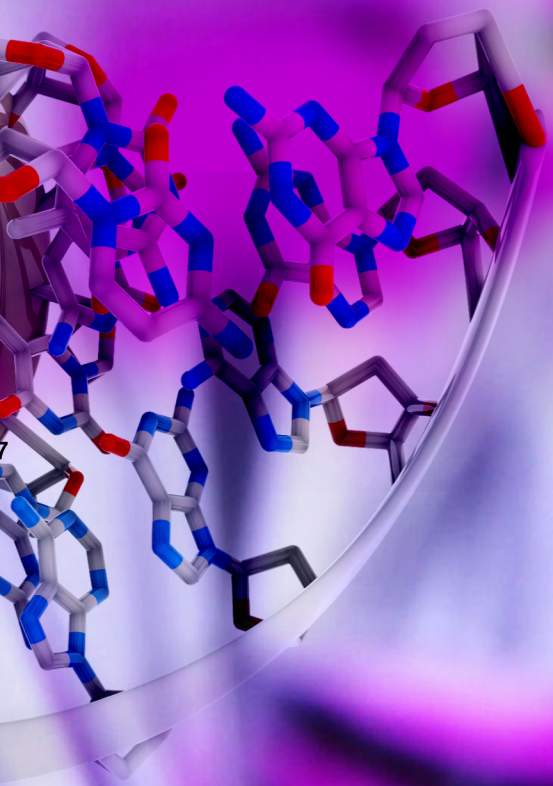
Topics in Anti-Cancer Research



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PREFACE

The tenth volume of **Topics in Anti-Cancer Research** covers new developments in the field of cancer. It comprises six comprehensive chapters covering exciting contributions in frontier areas of anti-cancer research.

Tehrani, in chapter 1, discusses the mechanism of anticancer activity of the peptides that can play a major role in combating cancer diseases. Rezaei and Rostami in chapter 2 of the book, present an appropriate model for investigating the possibility of chronic lymphocytic leukemia (CLL) control using fractal parameter. Veena *et al.* discuss the mechanistic insight of rhenium-based compounds as anti-cancer agents in the next chapter of the book. Veena *et al.*, in the fourth chapter of the book, provide insights on targeting cancer-specific inflammatory components in cancer therapeutics. Mandlik and Mandlik in the fifth chapter, discuss the anticancer potential of marine natural products in a diversity of flora and fauna, as well as their probable mechanisms of action. In the last chapter, Kajbafzadeh *et al*. address patient-derived xenograft (PDX) clinical trial designs in anti-cancer research.

I am thankful to the authors for their excellent contributions and to the reviewers for their in-depth and comprehensive comments for the improvement of the chapters. I am also grateful to Mr. Mahmood Alam (Editorial Director), Mr. Obaid Sadiq (Incharge Books Department), Ms. Asma Ahmed (Senior Manager Publications) and other colleagues for their support and assistance in the finalization of this volume.

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CHAPTER 1

Peptides Can Play a Major Role in Combating Cancer Diseases

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Abstract: Cancer diseases affecting many organs of human body have caused a major concern among the people all over the world. The conventional anticancer drugs, although have given some relief in the patient conditions, still cannot provide reliable treatment. Moreover, these drugs produce side effects in patients and in the worse cases, the problem of rising resistance phenomena against such drugs gradually put the patients' lives even in more serious situation. Therefore, identifying and introducing compounds with new identities to produce effective treatment with low side effects are highly demanded. Small peptides with anticancer activity have been shown to fulfill this demand. Peptides, with naturally or synthetic origin, have several advantages over common drug molecules such as low toxicity, low immunogenicity, amenable to several changes in their sequences and thus giving various homologues or analogues. Moreover, peptides in conjugation with heterocyclic active compounds and/or known anticancer drugs may result in molecules with new identities which show both benefits of individual components within their unit structures. In this regard, peptide conjugates may play a role, not only as anticancer agents but also as cell-membrane penetrating and/ or cell targeting agents to help direct cancerous tissue internalization of the known anticancer agents, and so, preventing or lowering the incidence of side effects of the anticancer drugs on healthy tissues. In this chapter on the basis of several experiments, information about various peptide categories, their analogues and conjugation with other bioactive compounds is given. The discussion is focused on the anticancer activity of peptides, those primarily known for other biological activities. Understanding the cause of these activities may help to find out and make clearer the mechanism of anticancer activity of the peptides.

Keywords: Anticancer, Bioactive compounds, Cell-membrane penetrating, Cell targeting agents, Peptides, Peptide conjugation.

INTRODUCTION

Cancer is defined as an uncontrollable growth and division of cells involving nearly every part of the body. The cancerous cells can even migrate and invade

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other organ tissues, which cause a phenomenon called metastasis [1]. Cancer metastasis involving many organs eventually leads patients to face death.

According to the World Health Organization (WHO) reports, it was estimated that 9.6 million deaths due to cancer occurred in 2018 and the incidence of cancer involved 18.1 million new cases worldwide and the cases will rise up to 29.5 million by 2040 [2]. In the world, 1 in 6 deaths occurs due to cancer [3]. Cancer can also cause enormous economic pressure along with immense social and emotional stress on patients and their families, especially in underdeveloped or developing countries with low-income people [4]. Cancer treatment with the conventional chemotherapy although shows effectiveness but presents side effects in healthy tissues of the body. The other important drawback of the chemotherapy regimen is the occurrence of drug resistance among cancer variants [5]. Peptides nowadays have been considered for application in many diseases and abnormal conditions including, infection, pain, inflammation, immune problems, diabetes and hypertension [6, 7]. Application of peptides has also been suggested in various cancers [8]. Compared with the conventional drugs, peptides have many advantages such as easily preparation, low toxicity and immunogenicity content, easily amenable to several changes in their sequences, good biocompatibility, high tissue penetration and low probability of raising resistance [8, 9]. The main disadvantage of peptides is their low stability against enzymatic lysis in gastrointestinal (GI) tract when they are administered for oral application. However, this unfavorable property of the peptides can be improved by employing several ways including peptide cyclization [10, 11], rearrangement of amino acid residues in the peptide chains [12], L-amino acids exchange by D-congeners [13, 14] and N- or C- terminal capping of the peptides in order to be stable against aminopeptidase or carboxypeptidase enzymes [10]. The N- or C-terminal modification of the peptides by different bioactive molecules may also be employed for several purposes or diseases, where such peptides are considered as conjugated peptides [15, 16] or hybrid peptides [10]. Peptides can be used not only as bioactive molecules by their own, but also as carrier of other bioactive agents for enhancing entrance into the target cells (thus, such peptides are so-called cell penetrating peptides, CPP) or targeting specific body organs with the aim of recognizing or even treating infected organs/tissues (so, the peptide are named tumor targeting peptides, TPP, or Radionuclide-Labeled Peptides) [17].

The main focus of this subject is on the use of peptides as anticancer agents in cancer research. Meanwhile, considering other activities of these peptides, the attempt is made to correlate such activities with anticancer properties of the peptides through which the kind of mechanism of anticancer activities involved may be deduced for the peptides. To organize the discussion, at first the characteristics of cancer cells will be overviewed. Several mechanisms of action

suggested for anticancer molecules will then be discussed. Different classes of anticancer peptides already designed and used for other biological activities, will make the other parts of this subject followed by summary and concluding remarks which come at the end.

Cancer Cells *versus* Normal Cells

The main characteristic of cancer cells is fast growing and dividing in an earlier and unusual time compared with normal cells, so that they make tumor (the mass of abnormal cells) which may often migrate from the initial place to the other parts of body and invade healthy tissues (metastasis) [9, 18]. On the other hand, normal cells grow and divide in time and remain wherever the body needs them. Normal cells need feeding for proliferation and therefore, new blood vessels are produced to afford this demand accordingly (angiogenesis phenomenon). Normal cells die whenever they are old or damaged in a programming manner (apoptosis phenomenon) or may be repaired when needed. Cancer cells, by capturing and employing angiogenesis mechanism, do not die and often survive in an unlimited time [19]. Accordingly, some functions of cancer cells become different from those of normal cells. These functions as related to the unblocked apoptosis are down regulation of apoptotic-induced proteins Bax and tumor suppressor protein p53, overexpression of matrix metalloproteinase 2 (MMP2), upregulation of the anti-apoptotic proteins Bcl-2, Bcl-XL, Bcl-Xs, and XIAP [20]. Apart from these different phenomena, size and shape of cancer cells are different from those of normal cells. Moreover, cell membrane in cancerous cells is characterized by phosphatidylserine (PS) exposed mainly outside of the membrane (outer leaflet), while in normal cells PS is buried inside the cell membrane along with phosphatidylethanolamine (inner leaflet) [4]. Since PS is a lipid with negative charge, it causes the cancerous cell surface becomes anionic, while zwitterionic phosphatidylcholine (PC) and sphingomyelin (SM) make an overall neutral charge in the normal cell surface (outer leaflet) [21]. Moreover, negative charge of cancerous cell membrane is potentiated by sialic acid attached- glycoproteins like mucins overexpressed in cancer cells [20]. In addition, proteoglycans containing glycosaminoglycan as side chains, bearing high negative charge (because of presenting many sulfate groups) are expressed differently in cancerous cells compared with normal cells [20]. It is also reported that the glucose metabolism ends with the higher secretion of lactate ions in cancer cells. Lactate ions, by neutralizing positive ions environmentally distributed, stabilize the negative charge of cancer cell membrane [22]. Also, interestingly, a greater number of microvilli structures are presented in cancerous rather than normal cell surface area [20]. This latter property increases the membrane surface of the cancer cells in favor of attracting higher concentration of cationic amphipathic molecules like peptides, comparatively.

CHAPTER 2

Studying of the CLL After Treatment Using Fractal Parameter of Neoplastic Lymphocytes Detection (λ_{nld})

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Abstract:

Background: Chronic lymphocytic leukemia (CLL) affects lymphoid cells and has a different chronic course. Some patients die due to the rapid progression of disease despite therapeutic measures. Therefore, it is necessary to identify, predict the disease, and seek new therapeutic strategies.

Introduction: Fractal geometry can be introduced as one of the most efficient methods to study the control of CLL in this type of cancer. The present research, presenting an appropriate model for investigating the possibility of CLL control using the fractal parameter.

Method: First, blood samples of the 30 healthy and 30 CLL samples with leukemia undergoing treatment were selected randomly. Second, the digital images were prepared using an optical microscope with a magnification of X100. Next, the fractal dimension of the lymphocyte nucleus of healthy and leukemia undergoing treatment was calculated using fractal software. Finally, the results were analyzed, and the fractal parameter of Neoplastic lymphocytes detection (λ_{nld}) was introduced and was calculated.

Result: The probability of CLL development increases with an increase in fractal parameter of Neoplastic lymphocytes detection (λ_{nld}). If the λ_{nld} value decreases during the CLL treatment, then the CLL was controlled. Full recovery occurs when λ_{nld} is smaller than the unit.

Conclusion: The average fractal dimension of the healthy lymphocytes and CLL nucleus and the fractal parameter of Neoplastic lymphocytes detection (λ_{nld}) in this res-

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earch were 1.781992 ± 0.046178, 1.801322 ± 0.042357 and 0.21833 respectively. Because the λ_{nld} is smaller than the unit, the full recovery occurs for this therapeutic group.

Keywords: B-CLL, Chronic, Computer-Assisted, Dimension, Fractals, Fractal Parameter of Neoplastic Lymphocytes Detection (λ_{nld}), Image Processing, Leukemia, Lymphocytic, Microscopy, Neoplasms, Recovery Process, Software.

INTRODUCTION

Cancer is currently demonstrated as a sophisticated and robust biological system [1]. Leukemia is an acute or chronic type [2]. The World Health Organization (WHO) introduced the protocols for leukemia classification [3]. Chronic lymphocytic leukemia (CLL) is the most common leukemia, and it has marked clinical, molecular, and prognostic heterogeneity characteristics [4 - 6]. Although some patients without treatment have a long life expectancy, others need efficient and rapid treatment from the beginning of the disease and may even die within a short period [7]. The absolute blood lymphocyte threshold for diagnosing CLL has been placed at >5000/μL B lymphocytes [8, 9]. The diagnostic evaluation of a patient suspected of having CLL should include a complete blood count with differential, the examination of the peripheral smear, and immunophenotypic analysis of the circulating lymphocytes. Bone marrow aspirate and biopsy are not required for the diagnosis of CLL. Most organs have fractal characteristics [10]. Cancer cells, in comparison to healthy cells, are not in a regular shape. Therefore, fractal geometry is a marker for the detection of cancer cells [11]. Fractal geometry plays a critical role in the computation of irregular surfaces. The term fractal, which was introduced by Mandelbrot in 1982, means irregular and rough shapes. The fractal dimension (FD) can be used for comparison of healthy and CLL blood cells. There are numerous technique for calculating the FD as below:

- Various step sizes measure the object quantities. The Log graph is plotted, and the slope of the data points is measured as a fractal dimension (FD).
- The box-counting method is a famous method for FD calculation.

This research was tried to investigate the detection and control possibility of CLL using a fractal parameter.

In the next, a historical review of leukemia types, diagnosis and treatment methods were initially discussed and in the second, the new method for CLL diagnosis and control was introduced.

Leukemia Types

Leukemia is divided into three types: Acute leukemia, chronic leukemia and lymphoma with a leukemic phase or chronic lymphocytic leukemia (CLL).

There are three different general types of chronic lymphocytic leukemia (CLL): B CLL, T CLL, and NK-CLL.

Diagnosis

Blood Tests for Diagnosis

The CLL diagnosis is done by minor modifications of the criteria [12, 13]. The morphology is used for distinguishing CLL from other types [14]. The absolute blood lymphocyte threshold for diagnosing CLL has been placed at >5000/μL B lymphocytes.

Image Processing for Diagnoses

The images of the blood marrow nucleus are extracted by a microscope .Then, the cells are classified as cancerous or non-cancerous cells. The experimental results show this method can diagnose the AML, ALL, and their types [15].

Image Acquisition

The image acquisition is very much significant to obtain an exact dataset. The blood smear microscopic image captured from a highly magnification microscope with high-resolution digital camera with high magnification near 1000X and constant magnification are essential in jpeg format. The captured images were reviewed for the identification of the type of blood cell.

Image Segmentation

The segmentation method is used to extract the nuclei from the blood cell images. Segmentation plays a major role in feature extraction and classification [16]. The proposed segmentation algorithm contains two parts; first, a cluster of nuclei is obtained by k-means clustering. Then, further objects in this cluster are neglected, and connected nuclei are separated. Here, it is used for segmentation with parameters: 4 clusters, Euclidean distance. Furthermore, the color information is represented by HSV color space. The object of every pixel is categorized into 4 clusters established based on H and S values, using cluster center and which it resembles the nucleus, background, and other [17].

CHAPTER 3

Mechanistic Insight of Rhenium-Based Compounds as Anti-Cancer Agents

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Abstract: Rhenium-based cancer drugs seem to be alternative drug candidates for platinum-based drugs in the treatment of cancer. Rhenium based anticancer compounds have attracted several researchers due to their various properties and wide-ranging applications as prodrug, drug conjugates, targeted delivery, imaging and cancer killing capacities. An array of rhenium compounds displays promising cytotoxic and phototoxic properties towards cancer cells. Re-complexes with aromatic or heteroaromatic ligands like polypyridine complexes, octahedral and tris (hydroxymethyl) phosphine(THP) have prodrug properties which upon irradiation, exhibits cytotoxicity activities. PentylcarbanatoRe(I) diimine complexes, 2-(acetyloxy) benzoate $Re(CO_3)$, $Re(CO_3)$ pentylcarbonato complexes, $[Re(CO)_3$(2-amino-4-phenyl amino-6-(2-pyridyl)-1,3,5-triazine)Cl] and Thiophene-2-carbohydrazide Re(V) complexes exhibits strong DNA binding activities. The 2-acetylpyridine-derived hydrazones $Re(CO)_3$, Re(I) polypyridyl complexes and *fac*-$[Re(CO)_3$(phen)] carboxy lato complexes were conjugated with aspirin reported as anti-inflammatory drugs. OxoRe(V) complexes with 3,3′-thiodipropanethiol tridentate ligands have been reported to inhibit the cathepsin B and K. Similar, Re-based complexes are synthesised using various ligands and that exhibit selectivity, controlled release and high efficacy potentials. However, still this research is at the preclinical studies. Re-based complexes have well-documented for antioxidant, drug delivery, selective anticancer activities, anti-inflammatory, DNA binding and damage inducing potential depending on the type ligand-Re complexes to contribute to cancer therapy. Thus, Re-compounds can be utilised in targeted therapies through coupling them with the biomolecules especially proteins and anticancer drugs. Among them, diselenium-rhenium complexes have selectivity and reduce the stress in the tumor environment to down regulate the breast cancer specific inflammatory cytokines to enhance the anticancer activity. Therefore, rhenium-based drugs are promising drugs candidate other than platinum-based drugs.

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Keywords: Anti-inflammatory, Anti-oxidant, Cancer imaging, Cancer treatment, Cytotoxicity, Mitochondrial targeting, Molecular target, Photoreactive, Rhenium drugs, Targeted therapy.

INTRODUCTION

Rhenium (Re) is a rare element with the atomic number of 75 and is not commonly found in humans. Although it is an expensive metal, it cheaper than platinum. Its electronic configuration (Xe $4f^{14}$ $5d^5$ $6s^2$) permits various oxidation states that support the diverse ligand types and coordination geometries [1]. Fine tuning the ligands in the metal environment has been shown to modulate its solubility, luminescence, cellular uptake and localization, biodistribution, toxicity, and pharmacological and toxicological outcomes. Complexes of Re(I) tricarbonyl subunits [$Re(I)(CO)_3$] are a promising group of anticancer compounds [3, 4]. In cancer biology, Re compounds have attracted the attention of researchers as they can be used in imaging owing to their luminescence or radioactive beta emitting isotopes, which can be combined by radio-imaging for cancer treatment. For example, ^{186}Re radioisotope (90 h $t_{1/2}$, 1.02 MeV, 137 keV) is used to treat patients with joint damage of the shoulder, elbow, wrist, ankle, and hip [5]. Furthermore, ^{188}Re (17 h t1/2, 2.11 MeV, 155 keV) is employed for the treatment of malignant tumors, bone metastases [6], and rheumatoid arthritis [7].

Several Re-based compounds exhibit antiproliferative activities and minimal toxicities. However, their mode of action is poorly understood. Re-based compounds have a wide range of oxidation states (-1 to +7), which enables the synthesis of diverse complexes that can be utilized for various applications. On the other hand, platinum-based and manganese-based compounds have a narrow range of oxidation states (+2 and +4) and exhibit high toxicity to the brain. Comparatively, Re is cheaper and has multimodal actions as well as diverse photophysical and photochemical characteristics that permit their use for theragnostic purposes [7 - 14].

RHENIUM BASED DRUGS IN CANCER IMAGING AND CANCER THERAPY

In addition to anti-proliferative activities, Re-based complexes are known to have luminescent properties that can be exploited for cell imaging through fluorescence microscopic techniques. Re-complexes with aromatic or heteroaromatic ligands of $Re(CO)_3$ polypyridine complexes and octahedral d_6 low-spin $Re(CO)_3$ complexes exhibit intense emission upon irradiation and exert cytotoxicity. These complexes can be conjugated as luminescent probes with biomolecules, thereby enhancing their solubility. PEGylated Re-complexes have been reported to accumulate inside the mitochondria of HeLa cells. Glycol-Re compounds have low cytotoxicity and

fast internalization, which is suitable or use as phosphorescent probes for cell imaging purposes. Water-soluble hexarhenium cluster complexes with benzotriazolate apical ligand possess excellent luminescence, with a half-life and high quantum yield that can be easily taken up by cells to illuminate them under UV irradiation without any cytotoxicity [14 - 18]. These imaging techniques constitute a new type of phosphorescent dyes.

Water soluble tris(hydroxymethyl)phosphine (THP) containing $Re(CO)_3$ complexes have excellent triplet-based luminescence, and their oxidative stress upon irradiation is 365 nm. These complexes display anticancer properties in human cervical (HeLa), ovarian (A2780), and cisplatin-resistant ovarian (A2780CP70) cancer cell lines only after irradiation. Ultrathin Re-disulfide nanosheets encapsulated with resveratrol and decorated with folic acid and bovine serum albumin (BSA) have been documented to release the resveratrol upon laser irradiation (808 nm) in HepG2 tumor-bearing nude mice. Re(I)/Au(I) luminescent bimetallic complex linked with the apical pyridine ancillary ligand through alkyne spacers exhibits antiproliferative activity upon irradiation [19 - 24]. Similarly, Re/Se and Re/Pt cluster complexes and aryldipyrrinato $Re(CO)_3$ complexes demonstrate potent photosensitizing potential by generating singlet oxygen under visible light irradiation [19 - 29].

MOLECULAR INSIGHTS OF ANTICANCER POTENTIAL OF RHENIUM-BASED COMPOUNDS

Rhenium-based complexes are known to exert several biological effects by intercalation, DNA binding activity, reduction of reactive oxygen species (ROS), enzyme inhibition, and interaction with signaling molecules and proteins (Table **1**). Re-based compounds, including PentylcarbonatoRe(I) diimine complexes, 2-(acetyloxy)benzoate $Re(CO_3)$, $Re(CO_3)$pentylcarbonato complexes, $[Re(CO)_3$(2-amino-4-phenylamino-6-(2-pyridyl)-1,3,5-triazine)Cl], and thiophene-2-carbo hydrazide Re(V) complexes, are known to form adducts in the major groove between the complexes and calf thymus DNA [9, 30]. Structure–activity relationship of Rhenium diimine complexes with different sulfonate and carboxylate ligands display *in vitro* anti-breast cancer activities through DNA binding [31]. Proteins appear to be the second most important target for Re complexes. Especially, Re(I) complexes and Re can covalently bind to histidine, glutamate, aspartate, and C-terminal carboxylate groups of peptides [32]. Binding interaction of Re(I) polypyridine complexes with BSA was found to decrease the α-helices, indicating the induction of conformational changes in the secondary structure of the protein [33].

CHAPTER 4

Targeting Cancer-Specific Inflammatory Components In Cancer Therapeutics

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Abstract: Cancer is a complicated family of diseases that causes major hurdles for global health. Several studies on cancer biology and cancer treatment strategies have revealed that cancer is highly genetically diverse and heterogenic in nature. The complexity of cancer is due to the highly inflammatory microenvironment which resembles wound healing process and highly acidic in nature. Hence, this condition is referred as cancer related inflammation (CRI) that drives the cancer resistance and subsequent recurrence of cancer after treatment. The major deregulated pathways associated with CRI are nuclear factor kappa B (NF-κB) and phosphoinositol-3-kinases (PI3-K) involved in cancer growth, proliferation, cancer cell survival and metastasis. Therefore, the protein factors of these pathways seem to be an attractive target for the molecular targetted therapy for cancer. However, efficient cancer treatment relies on the stages of cancer and the response to the treatment. Hence, cancer specific inflammatory components are the major targets for drug discovery, development and associated clinical trials.

Keywords: Anti-inflammatory, Cancer related inflammation, Cancer treatment, Cancer imaging, Cytotoxicity, Molecular target, Mitochondrial targeting, Photoreactive, Targeted therapy.

INTRODUCTION

Cancer is a disease of abnormally proliferative cells due to oncogenic events leading to genetic alterations. Cancers are genetic or induced by the events such as exposure to carcinogens, radiations, infectious agents and chemical agents. During the carcinogenic event, the mutated precancerous cells acquire ten hall-

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marks that include high expression of growth factors, inactivation of anti-growth signals, escape of cell death, high replication rate, angiogenesis, tissue invasion, inflammation, metastasis, metabolic adaptation and escaping the immune system. In addition to the above ten hallmarks, there are additionally enabled characteristics of cancer [1 - 4]. The first enabled hallmark of cancer is the inflammation-mediated tumour growth and promotion. The second emerging hallmark is the genomic instability and stress induced mutations that accelerate the proliferation and spread of cancer cells [5 - 10]. However, these multiple components of cancer are highly dynamic in nature that are multidimensional and interrelated to each other. These make them replicate than normal cells. Due to their high proliferative capacity, most cancer cells are anchor independent and can even survive without substratum and growth signals [6, 11, 12]. Therefore, many oncogenic signals convert normal cells by carcinogenesis, leading to abnormally proliferating cells by clonal expansion.

HISTORICAL PROSPECTIVE OF CANCER TREATMENT

Cancer is treated by surgery, chemotherapy, immunotherapy, targeted therapy, radiotherapy and palliative therapy [12 - 15]. Surgery and radiation therapy are the most widely-known earliest methods of cancer treatment with limited success rate in advanced stages of cancer up to 1960s. Surgery in combination with chemotherapy was able to increase the survival rate of the cancer patients. This strategy developed the combined treatment that had better outcome for cancer treatment. Radiation therapy was combined with other non-invasive treatments through advanced techniques in X-ray production. This was improved by imaging and computerised treatment [12, 13]. This approach is still in progress with advancement in imaging and diagnosis for cancer.

In early 19th century marks the beginning of cancer chemotherapy. Especially, in 1908 cancer treatment was most arsenicals were used. Later in 1943, nitrogen mustard was used to treat lymphoma patients. Around 1948, anti-folates were introduced. From 1951 to 1957, thiopurines and 5-fluorouracil were used in treatment. In 1958, methotrexate and antibiotics were approved for cancer treatment by FDA. In 1963, vinca alkaloids natural products were discovered and introduced in market. Imatinib and tyrosine kinase inhibitors were approved by FDA for the cancer targeted therapy 2005 onwards [13, 14]. Chemotherapy for cancer treatment is curative in some cancer such as Hodgkin's and non–Hodgkin's lymphoma, small cell lung cancer, ovarian cancer, choriocarcinoma, acute lymphoblastic and acute myelogenous leukaemia.

But due to the emergence of resistance for most of the used chemotherapeutic agents, the cancer treatment becomes ineffective. However, neoadjuvant

combined with chemotherapy treatment showed most effective survival rate in rectal, anal, bladder, gastroesophageal, breast, head and neck cancer patients [14]. However, the major limitations of conventional chemotherapy side effects included severe vomiting, stomatitis, alopecia and myelosuppression. This is because of non-specific nature of the therapy associated with side effects. Further, the recurrence nature of cancer is emerged to be as resistant and counter-productive to therapies [13 - 15]. Another important drawback is the rapid development of a different mechanism of drug resistance in cancer patients (Fig. **1**). Hence, therapeutic molecules that have cancer specificity can inhibit or kill the cancer cells seem to be attractive for cancer therapeutics with minimum side effects [16 - 21]. Due to these existing problems, the new therapy should be having multi-targets of cancer with long lasting and minimal secondary complications, which are in demand now [22 - 25].

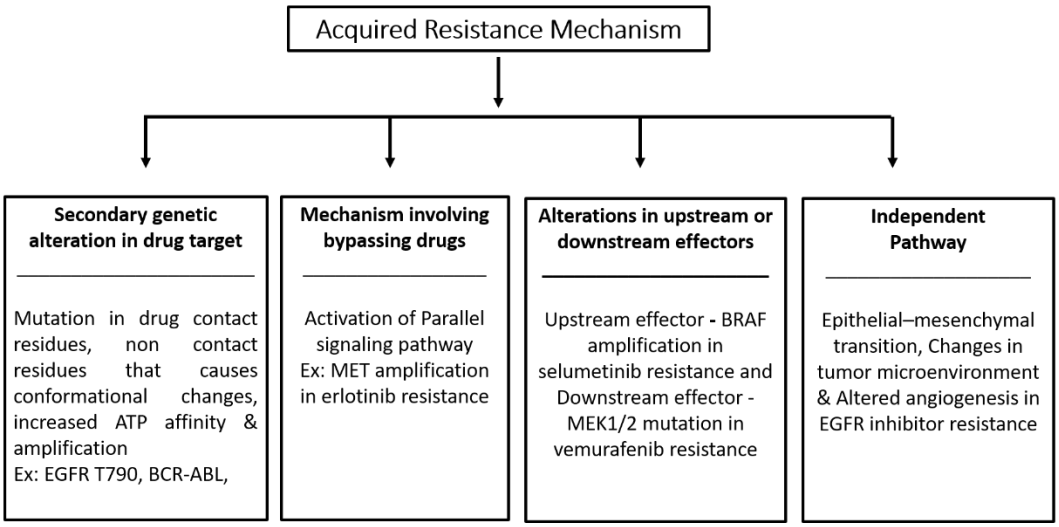

Fig. (1). Mechanism of resistances to the cancer treatments.

MOLECULAR BASIS OF CANCER AND MECHANISM OF CANCER RESISTANCE

During the oncogenic event, the oncogenic genes in the normal cells develop to precancer before advancing as cancerous cells. The oncogenic events such as microbial infections, carcinogens, radiation exposure and teratogens have been well studied for the initiation of cancer. These events eventually make sure that cancer cells acquire ten different capabilities that can distinguish them from the normal cells of the body. These hallmarks include high levels of growth signals, inability for antigrowth signals, escaping the cell death, limitless replication, invading the other tissues, angiogenesis, metastasis, inflammation, metabolic

CHAPTER 5

Marine Natural Products as a Source of Novel Anticancer Agents: A Treasure from the Ocean

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Abstract: One of the most deadly illnesses in the world remains cancer. New drugs with novel modes of action are urgently needed, recently, much work has been done on novel anticancer molecules derived from natural origins, particularly plants, microorganism and marine organisms. Marine natural products are repositories of novel bioactive metabolites containing different classes of bioactive substances and drug leads. This book chapter highlights the influence of marine organisms, with a specific focus on the ocean resources of marine plants, bacteria, algae, fungi, actinomycetes, sponges, soft corals, diatoms and ascidians, calculating above 90% of the overall ocean biomass. The cell lines and preclinical anti-cancerous effects of marine natural products were first introduced; their activity in preventing tumour development and associated compound-induced apoptosis and cytotoxicity was addressed. They are taxonomically distinct, having a high degree of efficiency and novel chemical structures that are pharmacologically active, creating tremendous potential for the progress of new anticancer molecules. These molecules have numerous pharmacological potentials, such as antioxidant, anti-tumour and anti-bacterial. Several marine anticancer agents have recently been extracted, characterized, described and are currently being studied for a clinical study. In this book chapter, we have attempted to assemble knowledge about the anticancer potential of marine products in a diversity of flora and fauna, as well as their probable mechanism of action. The molecular mechanisms that underpin the biological effects are also discussed. Finally, it addresses therapeutic methods and the present use of drugs extracted from the marine source, its future direction and limitations.

Keywords: Anti-Cancer, Bacteria, Bioactive Constituents, Corals, Marine, Marine Herbs, Microorganism, Natural Products, Seaweeds, Sponges.

INTRODUCTION

Cancer is a category of diseases in which specific cells of the body begin to divide incessantly and have the capacity to invade or spread to other areas of the body.

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The disease is becoming more prevalent due to changes in lifestyle, diet and global warming. Cancer treatment has advanced dramatically over the past two decades. Despite significant advances in understanding the nature of cancer, as well as its treatment and management, the illness remains to influence loads of people around the world. Cancer is related to a variety of causes, making recovery one of the most daunting jobs in medical science. According to a study published by the World Health Organization (WHO), a projected 12.7 million people were detected with cancer in 2008, with 7.6 million dying from it. According to this study, by 2030, there will be more than 21 million new cancer cases and 13 million deaths are predictable. Even though cancer accounts for about 13% of all deaths worldwide, more than 30% of cancer deaths can be avoided by adjusting or eliminating main risk factors [1].

Chemotherapy is used extensively in most cancer therapies. Chemotherapeutic agents lack selectivity, which means they can destroy healthy cells as well. They can also lead to multi-drug resistance. As a result of these issues, patients experience serious adverse effects, immune suppression, and poor clinical outcomes. Chemotherapy is causing a lot of problems, and scientists are working hard to solve them. Almost all chemotherapy medications on the market today, however, have significant side effects. Natural products are an important origin of potential novel drug candidates. Higher plants are thought to number at least 250,000 species worldwide, while marine organisms cover roughly 70% of the planet's surface. Due to defense, competition, or a variety of other environmental factors, both of these species can generate secondary metabolites with varying activities. Natural products and their derivatives account for more than half of all drugs used in clinical trials around the world. Higher plants account for at least a quarter of the total. Natural origin medications account for nearly 60% of cancer treatments approved by the FDA. Despite many innovations, many cancer management techniques are still a long way off from the real condition [2].

The marine milieu is regarded as a major source of masses of bioactive agents that can be used in a variety of fields, including medicine, food and technology. During the last 50 years, marine sources have produced main compounds that have demonstrated their ability for commercial production as functional foods, dietary supplements, enzymes and therapeutic agents [3, 4]. Broad varieties of plant-derived bioactive compounds are currently undergoing preclinical and clinical studies or are in progressive phases of development. While marine agents are currently unrevealed, it is expected that in the future period, the marine environment will be a prized reserve for forthcoming distinctive compounds because it encompasses 90% of the biosphere [5, 6]. Neuroprotective, antimicrobial, antimalarial, anti-inflammatory, immunomodulatory, anti-cancer and analgesic activities are abundant in the marine world, which includes a

diverse variety of aquatic animals and plants [7]. Alkaloids, peptides, terpenes, steroids and polyketides are the heavy biological composites found in the marine environment [8]. Surprisingly, marine-derived compounds and cancer therapy have always been related, as firstly identified marine-derived clinical drugs, Spongothymidine [9 - 11]. To date, a wide variety of marine-derived compounds are in different stages of clinical trials, and a significant number of constituents have been studied for preclinical experimentation.

Hence, the current book chapter aims to focus information on a numeral of marine natural agents, licensed medicines, derivatives, and other medicinal products that are currently undergoing anticancer clinical trials, as well as information on their molecular targets and current drug development challenges.

MARINE-DERIVED NATURAL PRODUCTS AND THEIR CLASSIFICATION

Marine organisms from the oceans are the huge source for the beginning of innovative anticancer drugs, as well as a collection of a massive chemical compound. For cancer therapy, various chemical mixtures of marine origin are extracted and incorporated using different procedures [12]. In either case, marine sources for anti-cancer therapeutic drugs are largely untapped. Regardless of how different classes have been constructed, the most unresolved marine-derived natural products contain compound structures. Peptides from various marine organisms, alkaloids from marine algae, terpenes with structural diversity, polyketides with intriguing biological activity, and high molecular weight organic sugars are all examples of compounds obtained from marine sources Table **1**. Both of these marine-derived natural products play a key role in the development of anticancer drugs, depending on their important natural functions against life-threatening infections [13].

Table 1. Chemical class, pharmacological activity and anti-cancer chemicals from various marine sources.

Chemical Class	Marine Source	Anticancer Molecules	Pharmacological Activity	Reference
Carbohydrates	Sponges and tunicates	Fucoidan, Heparin, Chondroitin 4 sulphate, Chondroitin 6 sulphate	Wound healing, Anti-mutagenic, Anti-cancer, Lipid-lowering; Anti-coagulant	[13]
Alkaloids	Sponges; Tunicates	Topsentin, Tambjamine D, Spongiacidin C	Anticancer, Cytotoxic, Anti-malarial, Anti-microbial	[13]
Peptides	Sponges	Brugine, Benzoxazolinone	Cardiotonic, Anti-viral, anticancer; anti-microbial	[14]

CHAPTER 6

PDX Clinical Trial Design in Anti-Cancer Research

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Abstract: Animal models are useful tools for understanding cancer biology and genetics and serve as an essential platform for the preclinical development of anti-cancer therapeutics. In this context, cancer-bearing patient-derived xenograft (PDX) models, also called cancer avatars, have successfully replaced the traditional cell line-derived models in recent years. PDX-based studies are now widely used for preclinical testing of novel treatments as well as tailoring personalized medicine. For anti-cancer research, however, the use of PDX models propagated from a unique patient does not fully represent the true therapeutic efficacy and toxicity of a drug. That is why many studies in this format later failed to show efficacy and safety in human clinical trials. Hence, the concepts of PDX clinical trials and co-clinical trials have gained importance and prospered in recent years. A PDX clinical trial implies investigation on a set of PDXs originated from multiple patients prior to an early phase human trial, whereas a co-clinical trial refers to drug response assays, in parallel and simultaneously with a human clinical trial, on a set of PDX models established from the same clinical trial participants. A carefully designed PDX- /co- clinical trial requires a meticulous calculation of the sample size, enrollment of pathologically and molecularly diverse patients, and selection of suitable endpoints and outcome measures. With a special focus on PDX clinical trial design in anti-cancer research, this chapter specifically addresses how to develop cancer-bearing PDX models, what to consider in characterizing them, how to track their fidelity to the parental tumor, how to estimate

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the number of animals included in a PDX trial, how to achieve greater power in the translation of final outcomes, what are the minimum endpoints to be considered, and what measures are preferred for evaluating the response to therapeutic interventions.

Keywords: Cancer Avatars, Clinical Trials, Malignant Neoplasms, Patient-Derived Xenograft Models, Xenograft Model Antitumor Assays.

INTRODUCTION

Continuous advancements in the understanding of cancer, especially the pathways critical for tumor generation and progression, have shed light on novel targets for the development of anti-cancer agents. However, to assess the safety and efficacy of a novel product in the pharmaceutical pipeline before entering into human trials, precise preclinical studies should be designed [1]. Following successful *in vitro* testing and confirmation of tumoricidal activity on cancer cell cultures, a new anti-cancer drug entity should undergo *in vivo* preclinical evaluations on appropriate animal models to delineate its efficacy, pharmacokinetics, pharmacodynamics, and toxicology [1, 2]. Historically, *in vivo* assessments of developing anti-cancer agents were carried out on murine models bearing transplantable murine tumors, and later on, human-originated cell-line derived xenograft (CDX) models [2 - 4]. Apparently, animal models with syngeneic tumors (isograft models) were proven not to be predictive for clinical response owing to molecular signature differences between animal-originated tumors and their relevant human-originated counterparts [2, 3]. In addition, the application of human-originated cancer cell lines for development of CDX models, is to become obsolete, because [5 - 12]: (1) the CDX models cannot fully represent the tumor heterogeneity, biomarker profile, and biological diversity of cancer patients; (2) the cell lines may become genetically-altered, because they are initially immortalized with specific genes to secure their capacity of unlimited proliferation, and so after several courses of culture and expansion, they may acquire genetic transformations and lose their fidelity to the original tumor; (3) the cell lines may gradually acquire homogenous phenotype after several subcultures, unlike the heterogeneous nature of their corresponding parental tumors, because the culturing condition allows most adapted cell components to remain; and (4) the tumor composition of traditional CDX models is dominated with cancerous cells but few cancer stromal cells, making them unsuitable for predicting a genuine response in clinical trials. For these reasons, only a limited number of anti-cancer agents studied on CDX models later thrived on reproducing efficacy in clinical trials. Therefore, from the beginning of the current century, particularly since 2010, the patient-derived xenograft (PDX) models have replaced the older versions in anti-cancer research [4].

PDX model is literally a representation of human cancer, where the tumoral tissue/cells (xenograft) are implanted or transplanted into an immunodeficient animal. Well-recapitulating the malignant tumors of humans, cancer-bearing PDX models, sometimes called "cancer avatars", may represent the complexity and the authentic nature of the original (parental) tumors and can be relatively reflective of the true response to novel anti-cancer therapeutics and personalized cancer treatments. Nonetheless, there still exist limitations in preclinical studies investigating the anti-cancer interventions on PDX models propagated from a unique patient or a limited number of patients, as they may not exhaustively predict clinical efficacy, given the heterogenous population of cancer patients. This is of particular significance considering that less than 10% of newly-developed anti-cancer agents, showing acceptable efficacy in preclinical *in vivo* studies, may ultimately demonstrate efficacy in oncology clinical trials and achieve regulatory approval [3, 4, 6]. To tackle this unresolved challenge, the concepts of PDX clinical trials and co-clinical trials have gained importance and prospered in recent years [4, 13]. With similarities in the lab work and management of the animal setting, a PDX clinical trial implies investigation on a set of PDX models developed from multiple patients prior to an early phase human trial, whereas a co-clinical trial refers to drug response assays, in parallel and simultaneously with a human clinical trial, on a set of PDX models established from the same clinical trial participants (Fig. **1**). *Via* creating a PDX repertoire of biologically-different cancer patients, PDX- /co- clinical trials are claimed to be more clinically translatable [4, 6, 14]. They, however, require more lab work, careful design, enrollment of pathologically and molecularly diverse patients, meticulous calculation of the number of PDX models and the number of animals per PDX model, and finally, selection of suitable endpoints and outcome measures. This chapter addresses these issues, with a special focus on PDX clinical trials investigating anti-cancer therapeutics.

RATIONALE FOR PRECLINICAL PDX-BASED STUDIES

There are two main purposes for conducting preclinical *in vivo* studies [15, 16]: (1) linking the drug discovery and *in vitro* testing to human trials, (2) determining the most effective treatment regimen for a patient in the context of personalized/precision medicine. However, in any case, the potential efficacy of the investigational drug or regimen should be initially evaluated *in silico* to avoid costly studies on animal models. To put it another way, some tumors are inherently resistant to certain targeted/immuno/chemo-therapeutics, and on this basis, performing a PDX-based study without in-depth *in silico* modeling and *in vitro* testing would be irrational. To this end, a multidimensional approach encompassing "omics" profiling and bioinformatics analyses might be required, which aids in identifying a set of potentially suitable drugs for a specific tumor

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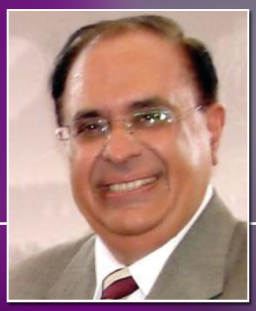

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