Frontiers in Clinical Drug Research

(Anti-Cancer Agents)

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PREFACE

Frontiers in Clinical Drug Research - Anti-Cancer Agents presents recent developments of various therapeutic approaches against different types of cancer. The book is a valuable resource for pharmaceutical scientists, postgraduate students, and researchers seeking updated and critical information for developing clinical trials and devising research plans in anti-cancer research.

The five chapters in this volume are written by eminent authorities in the field. Chapter 1 presents the latest research progress in the use of essential oils and monoterpenes as anti-cancer agents. Chapter 2 gives an overview of current innovative approaches to glioblastoma, focusing on the therapeutic benefits of immunotherapeutic agents and drug delivery systems. Chapter 3 summarizes the roles of ctDNA in clinical practice for diagnosis, treatment choices and responses to therapy in various solid cancers. Chapter 4 focuses on the anti-cancer effects of pitavastatin, including its mechanism of action as well as the potential adverse reactions linked to its clinical use. Chapter 5 discusses the biphasic role of cholesterol, in and around the cancer tumor, as a model target for anti-cancer therapeutic management.

I hope that the readers will find these reviews valuable and thought-provoking so that they may trigger further research in the quest for new and novel therapies against cancers.

I am grateful for the timely efforts made by the editorial personnel, especially Mr. Mahmood Alam (Director Publications) and Mrs. Salma Sarfaraz (Senior Manager Publications) at Bentham Science Publishers.

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CHAPTER 1

Essential Oils and Monoterpenes as Potential Anti-Cancer Agents

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Abstract: Cancer is a complex disease, and some projections indicate that in 2030, cancer mortality will reach approximately 11.4 million deaths worldwide. One treatment for cancer is chemotherapy. However, cancer cells could present resistance to the therapeutic compounds, and these compounds also have adverse effects. New drugs with anticancer activity have been successfully found in plants. Essential oils (EOs) are a mixture of over 100 volatile organic compounds abundant in aromatic plants. EOs are mainly composed of compounds of low molecular weight, such as monoterpenes, sesquiterpenes, and phenolic compounds. The chemical composition of EOs depends mainly on the plant species, place of origin, and climatic conditions. Generally, the EO density at room temperature is lower than that of water. They are brown, yellow, or colorless, and they have a perceptible aroma. EOs have been used throughout history in different areas, such as in foods, cosmetics, cleaning supplies, and traditional medicine for the treatment of certain health problems. Monoterpenes, built from two isoprene molecules, are hydrocarbon terpenes and oxygenated compounds (terpenoids), such as alcohols, aldehydes, ketones, acids, and esters. Monoterpenes are one of the main chemical constituents of EOs that have appeared in a large number of studies, and their anticancer efficacy has been documented between 2015-2020. This review presents the latest research progress in the use of EOs and monoterpenes as anticancer agents. The 115 EOs and 26 monoterpenes obtained from 36 different plant families included in this review show that Asteraceae and Lamiaceae have been the most studied families during this period.

Keywords: Antitumoral activity, Cytotoxic activity, Cancer, Cell lines, Essential oils, Monoterpenes.

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INTRODUCTION

Cancer is a disease with one of the highest rates of death worldwide. In 2018, 9.6 million deaths were attributed to cancer. Cancer is a disease with significant mortality and morbidity in all regions of the world. The mortality and incidence of cancer are rapidly increasing worldwide. The increase is associated with age and growing population and changes in the prevalence of the main risk factors for cancer [1].

The types of cancer with the highest number of deaths worldwide are lung, liver, gastric, colorectal, and breast cancer. In men, the most frequent type of cancer (the main cause of death) is lung cancer, followed by prostate, colorectal, liver, and stomach cancer. Additionally, in women, breast cancer is the most frequently diagnosed and is the main cause of death, followed by lung and colorectal cancer. Cervical cancer is fourth in incidence and mortality. However, the type of cancer with the highest diagnosis rate and the highest death rate varies between countries depending on economic development, social factors, and the lifestyle of the populations [2].

The main causes of cancer have been determined to be physical (ultraviolet radiation), chemical (asbestos, tobacco smoke, aflatoxins, and arsenic), and biological (viruses, bacteria, and parasites). Cancer development can also be caused by the hereditary genetic load of the body [3].

Cancer is characterized by the accelerated and uncontrolled growth of cells. Cancer defines malignant neoplasms characterized by metastatic growth [4]. It can occur in almost any organ or tissue in relation to a variety of etiological factors, such as genomic instability and environmental stress [4]. During the development of cancer, a multistep process is present, during which genetic alterations confer specific types of growth benefits; these alterations, therefore, drive the progressive transformation from normal cells to malignant cancer cells. Alterations in malignant cell growth are characterized by several key changes. Cells tend to alter their division cycle, presenting the following characteristics: sustaining proliferative signaling, evading growth suppressors, resisting cell death producing angiogenesis, activating invasion to other organs, metastasis, and enabling replicative immortality [5]. Cancer cells can generate a tumor, an irregular mass of tissue, which may or may not be solid, and can be differentiated into malignant or benign forms. Malignant forms can grow rapidly, invade and metastasize, and potentially cause death. There are several treatments of cancer, such as radiation therapy, surgery, hormone therapy, immunotherapy, and chemotherapy. These treatments can be used individually or concomitantly, depending on the stage of advancement of cancer [1]. Chemotherapy is a

treatment based on the use of drugs with cytotoxic activity, and in some cases, it can be applied before surgery in order to reduce the size of the tumor. Cytotoxic compounds can induce death in cancer cells, and the type of death that occurs is apoptosis [6]. Cell death by apoptosis aids in tissue homeostasis. This process is carried out by mitochondrial permeabilization and caspase activation. Chromatin condensation and DNA fragmentation are characteristics of apoptotic cells, which are ultimately eliminated by phagocytes. The dysregulation of apoptosis can contribute to pathologies such as cancer, autoimmune or neurodegenerative diseases [7].

More than 80% of chemotherapy drugs have been obtained from plants [8]. These drugs have shown activity against cancer. However, they also produce adverse effects, so alternatives are currently being sought for this disease. EOs and their different constituents have been a new source of cytotoxic and antitumor compounds. Currently, different *in vivo* and *in vitro* studies have been performed.

Many cultures have used EOs in religious practices, dating back to as early as 6000 BC. Later, ancient Egyptians used cypress, spikenard, and lotus oils in rituals and placed amphoras containing fragrant oils in the tombs for the voyage to the afterlife [9]. Additionally, they used EOs in medicine, perfumery, and cleansing. In Greece, visions of the oracles at Delphi came through inhalation of the smoke produced by Laurus nobilis [10]. The Greek and the Romans associated fragrances with their gods, but they also used EOs in baths and perfumery. The Persians made great contributions to the knowledge of aromatics and medicine. When the Crusaders returned to Europe, they brought perfumes, aromatics, and remedies unknown to Europeans before. Over the next few hundred years, the range of aromatic medicines became more popular, and the industry of EOs increased, providing oils for fragrance, flavor, and pharmaceutical purposes. In recent years, the therapeutic use of EOs has increased [11].

EOs are defined as highly concentrated aromatic oils of plant origin that are obtained from the leaves, flowers, barks, seeds, fruit peels, and rhizomes. EOs are a complex mixture of low molecular weight compounds, such as monoterpenes, sesquiterpenes, straight-chain aliphatic, aromatic, or heterocyclic compounds, and in some oils, allyl sulfides are found [12]. Many oils contain between a few dozen to 200 compounds. The chemical composition of EOs depends on many factors, such as the climate, season, geographical location, plant maturity, drying method, genetic variation, and stress during growth and storage. Thus, the variability in the EOs composition influences their properties [13, 14]. EOs are obtained by hydro or steam distillation [15], solvent extraction, supercritical fluid extraction [16], or cold pressing process [17].

CHAPTER 2

A Glance at Drug Delivery Systems and Emerging Immunotherapeutic Strategies for the Treatment of Glioblastoma

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Abstract: Glioblastoma (GBM) represents the most common and aggressive primary brain tumor with a 5-years survival rate lower than 10%. GBM worldwide incidence is about two to three per 100000 adults per year, and the standard treatment encompasses surgical debulking with subsequent radiation therapy and concomitant chemotherapy.

Given its heterogenicity, its intracranial location and the onset of multidrug resistance mechanisms, new tailored approaches, such as immunotherapy and drug delivery systems, have recently gained increasing interest. In recent years, tumor microenvironment exploration has revealed that immune response evasion is one of the crucial GBM diagnostic hallmarks. Since this discovery, the possibility to reverse tumor-mediated immunosuppression has received increasing attention, changing the paradigm of cancer treatment from chemotherapy to immunotherapy. On the other hand, the blood-brain barrier (BBB) represents the main challenge in developing therapeutics for central nervous system (CNS) tumors; for instance, 100% of large molecules and 98% of small molecules fail to achieve sufficient therapeutic doses at the brain. To this purpose, nanotechnology-based drug delivery systems represent promising platforms to improve drug bioavailability, reduce side effects and allow the co-delivery of multiple drugs to the target cells.

This chapter gives an overview of current innovative approaches to GBM focusing on the therapeutic benefit of immunotherapeutic agents and drug delivery systems. In particular, we aim to provide a summary of the recent clinical trials using immunomodulator, immune checkpoint inhibitors (ICIs), *i.e.* monoclonal antibodies (mAbs) blocking cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) and programmed cell death-1 (PD-1/PD-L1a), vaccination therapy. Although ICIs and vaccines have shown limited efficacy when used as monotherapy, promising results have been reported for their combination with immune adjuvants, such as chemo,

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Atta-ur-Rahman (Ed.)

radio- and photodynamic therapies, suggesting an emerging strategy for a more successful antitumoral immune response. With this chapter, we aim to provide an overview of the state-of-the-art and future perspective of GBM treatment, mainly addressed to pharmaceutical students and researchers in the field of clinical drug research.

Keywords: Blood-Brain Barrier (BBB), Blood-Brain Tumor Barrier (BBTB), Clinical trials, Combination approach, Drug Resistance, Glioblastoma, Immune Checkpoint Inhibitors (ICIs), Immunotherapy, Long-term Immune Memory, Nanocarriers, Nanomedicine, Vaccine.

INTRODUCTION

Glioblastoma (GBM), accounting for 54% of all gliomas and 16% of all primary malignant tumors of central nervous system (CNS), is the most common and aggressive brain tumor in adults [1]. The median age for the development of GBM is 64 years, but it can occur at every age, including childhood; most GBM are primary or *de novo* tumors without a known precursor, characterized by poor prognosis and most common in older patients (Fig. **1**) [2].

Despite advances in surgical techniques, radiation therapy, chemotherapy, and new therapies based on targeted agents and immune modulators, patients' survival remains low, with a 5-year survival rate lower than 10% [1, 3]. Several factors might influence GBM prognosis, such as the extent of surgical resection, tumor molecular classification, patients' age and Karnofsky Performance Status (KPS) at diagnosis. Specifically, lower age and higher KPS bestow more prolonged survival. Moreover, tumors greater than 5-6 cm at diagnosis, and those crossing the mid-line have been associated with negative outcome, whereas cerebrum and cerebellar tumors that are more accessible with surgery, lead to better prognosis [3, 4].

Aside from age, which is known to play a crucial role, and the exposure to ionizing radiations, responsible for a limited number of cases, GBM's risk factors are overall poorly established [5 - 7]. Only less than 1% of GBM is associated with hereditary cancer syndromes, such as Turcot syndrome, Li-Fraumeni syndrome, tuberous sclerosis and neurofibromatosis types 1 and 2 [8]. Environmental exposure, infections, trauma, smoking, cell phone use, and pollution do not seem to be involved in GBM development [9, 10].

The association between GBM incidence and specific genes, accounting for nearly 27% of familial risk [3], has been investigated by the Cancer Genome Atlas project (TCGA), which showed that most GBM present activation of three different pathways, namely retinoblastoma, tumor suppressor p53 and receptor

tyrosine kinase/Ras/phosphoinositide 3-kinase signaling pathway [3, 4, 11]. Importantly, future transcriptome-wide association investigations, together with molecular epidemiology studies, might unravel new targets for GBM treatment, predictors of side effects, and prognostic markers, contributing to better management of this disease.

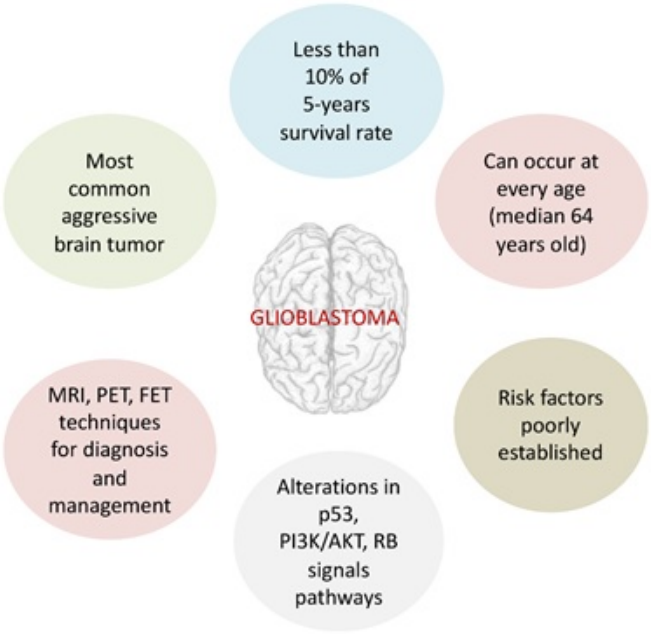

Fig. (1). GBM general characteristics.

Based on histologic diagnosis, GBM is characterized by increased cell density, atypia, areas of necrosis, and robust angiogenesis [12, 13]. Furthermore, GBM has a strong brain tropism and only rarely disseminates into organs, probably due to the metabolic adaptation and immunologic peculiarities of the brain microenvironment [14 - 16].

GBM is classified as primary or *de novo* tumor without a known clinical precursor, or secondary GBM, which is most often considered as the progression/transformation of lower-grade gliomas. In 2016, the WHO revised brain tumors classification incorporating tumor genetic alterations to the classic histological ones [12]. Therefore, circumscribed gliomas (grade I) were classified as mostly benign and curable by complete resection, while gliomas from grade II to IV result more diffuse and infiltrating, thus very rarely cured by resection (Fig. **2**) [17].

CHAPTER 3

ctDNA in Solid Tumors: Role in Diagnosis, Prognosis and Treatment

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Abstract: Solid tumors have long been known constantly to shed many biomolecules such as DNA, RNA and proteins into the blood and other body fluids. These biomolecules can circulate in the blood free of cells or by binding to proteins or lipids. Circulating tumor DNA (ctDNA) is tumor-derived cell-free DNA (cfDNA) and is often confused with non-tumor derived cfDNA. Recent advances in laboratory techniques enable better capture and analysis of trace amounts of circulating materials. Liquid biopsy is a minimally invasive method and allows analyses of circulating tumor cells released from peripheral tumors and/or metastatic tumors and nucleic acids in the cell-free circulation, in particular ctDNA, microRNA and extracellular RNA. The fact that ctDNA completely reflects the tumor genome has made it a powerful clinical and research tool in liquid biopsy, and a number of studies have been conducted on the diagnostic, predictive and / or prognostic use of ctDNA in cancer over the last few years. There are studies confirming the clinical validity of ctDNA for detecting tumor heterogeneity and resistance mutation, identifying candidates for targeted therapies, disease monitoring and therapeutic response assessment, early detection of recurrence, monitoring tumor burden, and risk classification. In this chapter, we have summarized the roles of ctDNA in clinical practice for diagnosis, treatment choices and responses to therapy in various solid cancers.

Keywords: Breast cancer, Cell-free DNA, Circulating tumor cells, Circulating tumor DNA, Colorectal cancer, Head and neck cancer squamous cell carcinoma, Liquid biopsy, Non-small cell lung cancer, Prostate cancer gastrointestinal stromal tumor, Solid tumors.

INTRODUCTION

Cancer diagnosis and treatment are based on the phenotypic or genotypic features of the cancer cells and potential treatment is possible with early diagnosis. Solid biopsy, an integral part of the tumor, is often needed for diagnosis, staging, and monitoring of disease progression and establishing effective treatment for cancer

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patients, but has several drawbacks, which are bleeding, nerve injury or the risk of spreading the disease. In heterogeneous tumors (*e.g.*, breast tumors), tissue biopsy may not represent the entire tumor, and for tumors in hard-to-reach regions (*e.g.*, such as lung, breast and pancreatic cancers), obtaining a solid biopsy can be painful for the patient and may cause death. Therefore, obtaining cancer cells using non-invasive methods with a low risk of complications is advantageous for both the patient and the physician. When the metastasis process is effectively interfered with, effective cancer treatment can be achieved.

The main characteristic of malign tumors is their ability to metastasize. The clinical appearance of metastasis in distant organs is the final stage of cancer and is the cause of most cancer-related deaths [1]. The basis of metastasis biology is that the tumor cells show a tendency to transfer from the primary region into distant organs where the cells can remain asleep until they begin to regrow [2]. These cells are called circulating tumor cells (CTCs). In cancer patients, blood may contain other analytes besides CTCs, such as circulating tumor DNA (ctDNA), which is released from tumor cells and could be single or double-stranded and cell-free DNA (cfDNA) which includes DNA fragments from normal cells and extracellular vesicles (exosomes) [3].

Considering the generally accepted cancer models, the spread of cells from the primary cancer region into the circulation can be considered as an advanced stage, but it has been shown that cancer starts releasing neoplastic cells into the bloodstream before they reach the metastatic stage, and even earlier [4, 5]. However, cancer patients have more fragmentation of DNA than healthy individuals [6].

In 1869, pathologist Thomas Ashworth noticed CTCs in the blood of a metastatic cancer patient and turned them into what is now called liquid biopsy (Table **1**) [7 - 20].

Table 1. Landmarks in the detection of liquid biopsy development.

1948	1977	1989	1994	1996	1997	2005	2012-2013	2014	2017	2019-2020
cfDNA was first reported in human	Leon *et al.* found increased cfDNA	Stroun *et al.* first reported the appearance of	Sorenson *et al.* were detected a mutated RAS gene fragment	Nawroz *et al.* were first reported the detection of microsatellite	Lo *et al.* were reported the discovery of the cell-free	Diehl *et al.* were studied detection and	Attention to the detection and follow-up	First clinical validation of the detection	ctDNA analysis kits started to be	ctDNA analysis could be a potential

(Table 1) cont.....

1948	1977	1989	1994	1996	1997	2005	2012-2013	2014	2017	2019-2020
plasma by Mandel and Metais [7].	levels in the serum of cancer patients [8].	in plasma of cancer patients [9].	in the blood plasma of pancreatic cancer patients [10].	instability (MSI) and loss of heterozygosity (LOH) in cfDNA [11].	fetal DNA (cffDNA) in maternal plasma [12].	quantification of mutations in the plasma of patients with colorectal tumors [13].	ctDNA in the tumor [14 - 17].	of mutations from ctDNA by Thiery *et al.* [18].	used for oncology research [19].	alternative to invasive tissue biopsies [20].

Liquid biopsy aims at intimately observing the progress of cancer or treatment efficacy with minimal risk and burden for the patient and physician. The liquid biopsy may enable detailed profiling of tumor and early detection of a relapse with the isolation of CTCs and ctDNA (Fig. **1**).

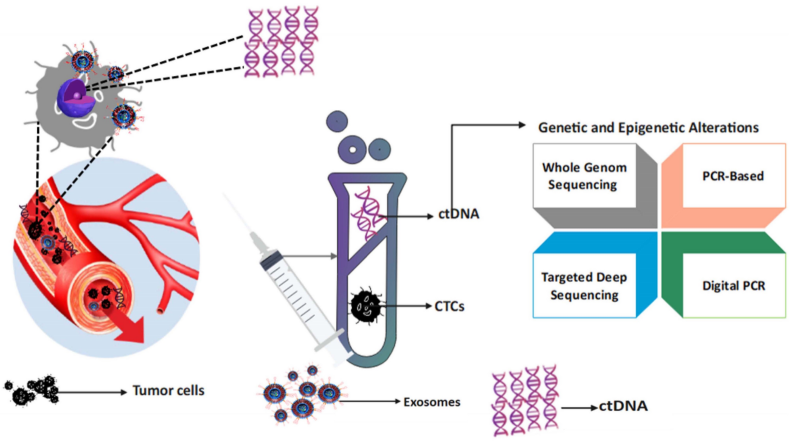

Fig. (1). An overview of blood-based liquid biopsy. In cancer patients, blood contains CTCs, ctDNA, exosomes. The liquid biopsy may enable detailed profiling of tumor and detection of a relapse relative early with the isolation of CTCs and ctDNA.

The analysis of ctDNA by different detection methods has an important role in the follow-up of patients, especially those diagnosed with cancer, and who have certain genetic markers. However, these methods have both advantages and disadvantages (Table **2**).

Table 2. ctDNA detection methods.

Methods for Detection	Technique	%ctDNA	Application	Advantages	Limitations
Digital PCR [16, 21 - 24]	Droplet-based digital PCR Microfluidic digital PCR	~0.01%	Known point mutations	High sensitivity	Only can detect specific genetic loci and known mutations

CHAPTER 4

Pitavastatin and Cancer: Current and Future Prospects

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Abstract: Pitavastatin is a synthetic 3-hydroxy-3-methyl glutaryl coenzyme A reductase inhibitor, which was approved for the primary treatment of hypercholesterolemia and combined dyslipidemia since 2009. Today, *in vitro* and *in vivo* studies have shown pitavastatin as a potentially effective therapeutic agent for different cancers, including; liver, ovarian, breast, skin, and intestinal cancers. These studies have evaluated pitavastatin both as a single treatment and in combination with other therapeutic options. This chapter focuses on the potential anti-cancer effects of pitavastatin, including its mechanism of action as well as the potential adverse reactions linked to its clinical use.

Keywords: Anti-cancer, Anti-inflammatory, Apoptosis, Autophagy, Cell cycle arrest, Pitavastatin.

INTRODUCTION

Cancer is one of the deadliest diseases and accounts for 13% of total deaths worldwide [1]. It is a multifactorial disease that is unlikely to be treated with a single treatment. Surgery is the primary strategy for treating many cancers. Other forms of cancer treatments include radiation therapy, hormone therapy and chemotherapy. Unfortunately, despite the enormous efforts invested to treat cancers, there has been limited success because of the undesired side effects and drug resistance induced by the aforementioned treatments. Most of the current cancer treatments have been shown to kill cancer cells by activating apoptotic cell death [1]. Apoptosis is mainly executed by two caspase-dependent pathways: the extrinsic pathway and the intrinsic pathway.

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Both pathways depend on a group of signaling pathways that regulate the programmed cell death process. However, cancer cells usually develop several mutations or alterations to avoid apoptosis. While apoptosis has generally been considered as the principal mechanism of cell death induced by cancer treatments, it has become obvious that it can also induce autophagy. The later cellular process is a degradation mechanism involving the recycling of cellular organelles and macromolecules. Autophagy is usually activated under conditions of stress to promote cell survival. There is, however, a debate as to whether the activation of autophagy in response to chemotherapeutic agents enhances or inhibits cell death [2].

Statins, a group of drugs that reduce plasma cholesterol levels, have been shown to induce both apoptosis and autophagy [3]. They were initially developed as treatments for hypercholesterolemia and to manage coronary heart disease [4]. These drugs block the cholesterol biosynthesis pathway by inhibiting the rate-limiting enzyme in the mevalonate (MVA) pathway, 3-hydroxy- 3-methylglutaryl-CoA reductase (HMGCR) (Fig. **1**).

Statins are categorized into two major classes: the natural products-based statins (mevastatin, lovastatin, pravastatin, simvastatin) and the synthetic statins (fluvastatin, atorvastatin, cerivastatin, pitavastatin, rosuvastatin) [4, 5]. Structurally, statins comprise of two parts: the pharmacophore moiety (either being carboxylic acid chain in the active form or a lactone ring in the prodrug) that is responsible for the inhibition of HMGCR, and the ring system moiety that is different for each type [4]. While most statins are administered orally as actively opened rings, lovastatin and simvastatin are administered as lactones that are activated in the body [4, 6]. However, statins differ in their pharmacological effects, lipophilicity, hydrophilicity, kinetic profile, rate of metabolism and the formation of active and inactive metabolites. Myotoxicity and rhabdomyolysis are the most severe undesired effects of statins [7, 8].

The anti-cancer properties of statins have been demonstrated in monotherapy and in combination therapy with other chemotherapeutics [9 - 11]. Different statins such as simvastatin, lovastatin, mevastatin, fluvastatin, and pitavastatin have been shown to induce apoptotic cell death in a subset of *in vitro* and *in vivo* cancer cell models [9, 12 - 16]. Recently, pitavastatin gained more attention for the prevention and treatment of cancer, as it can inhibit cell proliferation, inflammation, tumor initiation, and progression and inducing different modes of cell death [17 - 20].

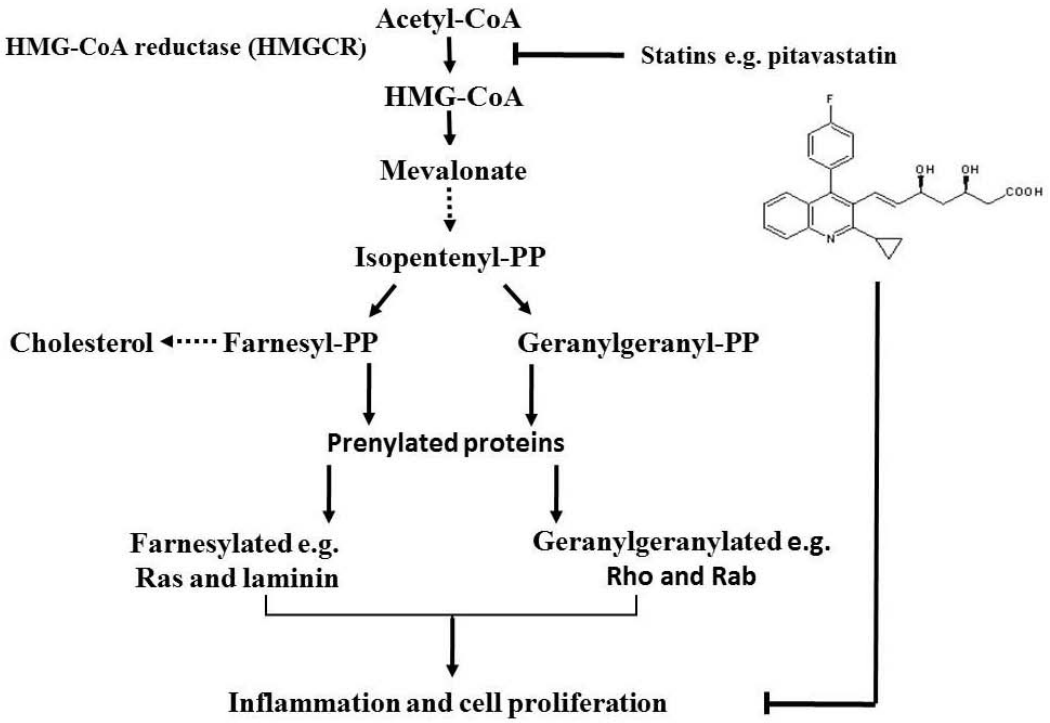

Fig. (1). Schematic illustration of the mevalonate pathway and its implications on inflammation and cell proliferation. Sites of action of HMG-CoA reductase inhibitors (statins, *e.g.*, pitavastatin) are indicated.

Pitavastatin has been shown to exert potent cytotoxic effects on glioblastoma growth *in vivo* [16]. The mechanism of action of pitavastatin includes up-regulation of the cell cycle regulator p21 and inhibition of NF-κB, which resulted in cell cycle arrest and apoptosis [21, 22]. Interestingly, autophagic cell death was also shown to be induced by pitavastatin [19]. Finally, recent findings showed that pitavastatin and dacarbazine synergistically inhibit melanoma cell survival by inducing cell cycle arrest, apoptosis, and autophagy [15].

The objectives of this chapter are: 1) review the available preclinical and clinical literature focused on the potential efficacy of pitavastatin drugs in cancer treatment, and 2) discuss the molecular mechanisms driving its anti-cancer effects. This chapter also offers insights into the possible future directions of pitavastatin.

PITAVASTATIN: THE CHEMICAL COMPOUND

Pitavastatin, (+)–monocalcium bis (3R,5S,6E)-7-[2-cyclopropyl-4-(4-fluoro-phenyl)-3-quino- lyl]-3,5-dihydroxy-6-heptenoate] (M.W. 880.98) is a synthetic

CHAPTER 5

Cholesterol: A Potential Target for Intervention in Anti-Cancer Therapy

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Abstract: Being polycyclic hydrocarbon, cholesterol has the quality for making DNA adduct within cell nucleus. Structurally cholesterol and its epoxide are very close to polycyclic carcinogenic precursor *e.g.* benzo-alpha-pyrene, which is well known for its carcinogenic pulse by forming adduct to chromosomal DNA. In fact normal cross membrane transports of cholesterol, either on cell surface or on nuclear membrane turn desynchronized in cancer tumor cells. A cholesterol loaded cell nucleus has been correlated to wobbly cell cycle operation and aberrant cell proliferation in many cancer type *viz.* leukemia, breast carcinoma, prostate carcinoma *etc.* A reprogrammed cholesterol metabolism affects tumor associated immune cell activity, cellular apoptosis and kinetics of cell survival. In fact, cholesterol concentration within cell nucleus has been found correlated with cellular life span. In tumor microenvironment intracellular cholesterol concentration varies from one to another cell type. While it increases within tumor cells, the surrounding immune cells die because of scarcity of intracellular cholesterol concentration. Intervention of this biphasic role of cholesterol in and around the cancer tumor could be a model target for anti-cancer therapeutic management.

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INTRODUCTION

In a recent report, it has been emphasized that atherosclerosis and carcinoma are two different episodes of dysfunctional cholesterol homeostasis [1]. While in one hand, cholesterol is a part of essential commodities used for maintaining cellular metabolism [2], membrane scaffolding [3, 4] and epi-chromosomal ventures [5]; on the other hand, cumulative accumulation of cholesterol, either in extra- or intra-cellular edge, is injurious to health. While an atherosclerotic association of cholesterol in cardiovascular diseases (CVD) and stroke is a well-known feature for a long time due to its excess deposition and adherence in the intima of blood vessels, an upcoming concurrence of message on over-accumulation of cholesterol in intracellular domain, either in the cytosol or cell nucleus, is now targeting the oncogenic affair of cholesterol in a biological system.

Intracellular cholesterol homeostasis is maintained from the balanced activity of three-way operations. These are 1) LDL receptor (LDLR) mediated influx of extracellular cholesterol, 2) Synthesis of cholesterol within cells by the activity of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) and 3) Efflux of cholesterol by ATP-binding cassette (ABC) transporter proteins such as ABCA1 and ABCG1 [6, 7]. Again, LDLR activity is regulated by various factors, *e.g.,* its inactive existence in a bound state with insulin receptor [8], feedback regulation by sterol regulatory element-binding proteins (SREBPs) [9], and post-transcriptional regulation by PCSK9 [10]. Genetic heterogeneity guides the potentiality of the major cholesterol routes [6, 7]. An imbalance of the equilibrium in these processes predates carcinogenesis [11, 12]. In various types of cancer, hyper-expressions of active LDLR and HMGCR have been documented [6, 13]. Cholesterol rich lipid rafts or microdomains [14, 15], especially on nuclear surface membrane, have been found to coordinate in organizing the signalling molecules and transduction of signals [16] for cell proliferation within tumor tissue.

The pivotal role of cholesterol, within membrane lipid microdomains of the cell nucleus, on transcription regulation to rule proliferation, differentiation and apoptosis with the alterations of the chromosomal lipid-protein complex has been recorded in several reports since the early decade of the 21st century. Recent works also have monitored the impact of LDL transmitted cholesterol through LDLR on protein expressions in cell cycle operation [17, 18] and cancer cell

progression [19 - 22]. The entry of cholesterol from the cytoplasm to the cell nucleus is expected through the peripheral-type benzodiazepine receptor (PBR) situated on the nuclear surface membrane [20 - 24].

Reprogrammed cholesterol metabolism, either by cell-intrinsic or cell-extrinsic signal, in the tumor microenvironment (TME) cumulatively generates a convenient home for aggressive tumorigenesis. Cholesterol or cholesterol-derived polycyclic metabolites play intense roles in cancer cell progression by suppressing anti-tumor immune responses [25 - 27]. In pre- and post-TME, the immune cells die, maybe because of their insufficiently low intracellular cholesterol level [25, 26]. PI3K/AKT (phosphatidylinositol 3-kinase/protein kinase B) signalling, a dynamic regulator of cell proliferation, growth, survival, migration and metabolism, can up-regulate genes in the cholesterol pathway and found hyperactive in carcinoma tumors [28]. *MYC,* a viral oncogene, can up-regulate the mevalonate pathway [29]. Repression of LXRα signalling by hyperexpression of oncogene c-Fos elevates the production of cholesterol and cholesterol-derived oxysterols and bile acids [30], which in turn increases inflammation and carcinogenesis. Tumor suppressor p53 upregulates the cholesterol-efflux transporter ABCA1 to prevent excess cholesterol accumulation within the cells, and suppression of the mevalonate pathway by statins appears to effectively retard tumorigenesis in p53 loss [31]. Thus activities of tumor suppressors are also correlated with the metabolome of cholesterol homeostasis. In TME, low-pH-responsive genes are also associated with cholesterol homeostasis and inversely correlated with patient survival [32].

Lastly, cholesterol homeostasis is riddled between the pathology of carcinoma and atherosclerosis. In this context, the pleiotropic effects of statins drugs are in major focus. While statins are the marvels for taking care of atherosclerotic diseases like heart attack and stroke by inhibiting intracellular cytosolic enzyme HMGCR [33], the therapeutic potential of statins in cancer treatment [12] is also challenging ground. Inhibition of isoprenoid synthesis by statins can suppress down the mevalonic acid pathway, and its mechanism of action beyond cholesterol reduction (pleiotropic effects) [34], *i.e.,* plaque stabilization and improvement of endothelial functions, anti-inflammatory and anti-oxidative effects have attracted interests for its use in cancer therapy. But the reports from different sets of clinical trials on statins with various cancer phenotypes are now flummoxed with the school of mixed thoughts. In fact, statins and other cancer drugs are mystified in their mechanism of action to discriminate atherosclerosis and carcinoma from the perspectives of intracellular cholesterol homeostasis. These issues will be discussed in more detail in the following sections.

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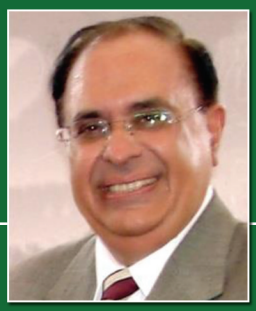

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Prof. Atta-ur-Rahman, Ph.D. in Organic Chemistry from Cambridge University (1968) has 1,232 international publications (45 international patents and 341 books). He received the following awards: Fellow Royal Society (FRS) London (2006), UNESCO Science Prize (1999), Honorary Life Fellow Kings College, Cambridge University (2007), Academician (Foreign Member) Chinese Academy of Sciences (2015), Highest Civil Award for Foreigners of China (Friendship Award, 2014), High Civil Award Austria ("Grosse Goldene Ehrenzeischen am Bande") (2007), Foreign Fellow Chinese Chemical Society (2013), Sc.D. Cambridge University (UK) (1987), TWAS (Italy) Prize (2009). He was the President of Network of Academies of Sciences of Islamic Countries (NASIC), Vice President TWAS (Italy), Foreign Fellow Korean Academy of Science & Technology, President Pakistan Academy of Sciences (2003-2006) and (2011 – 2014). He was the Federal Minister for Science and Technology of Pakistan (2000 – 2002), Federal Minister of Education (2002) and Chairman Higher Education Commission/Federal Minister (2002-2008), Coordinator General of COMSTECH (OIC Ministerial Committee) (1996-2012), and the Editor-in-Chief of Current Medicinal Chemistry.