Application of Traditional Chinese Medicine in Cancer Treatment

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Abstract

Cancer remains a leading cause of death in the world. Traditional medicinal herbs have been confirmed to have the character of anticancer. Besides cancer cell-killing activity, herbal medicine can also be used to strengthen the immune system and to ease the side effects of conventional cancer therapies. Based on the literature, there are three stages of herbal medicine studies in cancer treatment. The first stage herbs are those, which have been demonstrated to have anticancer activity from in vitro cell line study or animal model study. However, the active compounds with anticancer activity in those herbs have not been clearly identified. The second stage herbs are those, whose potential anticancer compounds have been identified. The unresolved question is to understand their detailed molecular mechanism. It is important to identify their molecular targets. The well-known anticancer agents camptothecin from Camptotheca acuminata, paclitaxel from Taxus chinensis, homoharringtonine from Cephalotaxus harringtonia and indirubin from Baphicacanthus cusia have been used in the treatment of solid tumors or leukemia. Camptothecin, paclitaxel, homoharringtonine, and indirubin can kill cancer cells by inhibiting the normal functions of topoisomerase I, microtubule, ribosome and cyclin-dependent kinase respectively. They are considered as the third stage herbs because their in vivo molecular targets have been identified. Despite the clinical success due to the pharmacological unique of these active constituents, there still exist problems with these anticancer drugs regarding chemical properties, target binding affinity and specificity. In the review, we also discuss importance and limitations of computational calculations in drug development with the focuses on medicinal herbs. In conclusion, anti-proliferative activities of medicinal herbs may not be specific and effective for cancer treatment. However, there are needs to identify bioactive compounds in medicinal herbs, especially those proved to be effective in clinical practice.

Introduction to traditional Chinese medicine in cancer treatment

Challenges in cancer treatment

Cancer has traditionally been considered as a disease of genetic defects such as gene mutations and deletions, as well as chromosomal abnormalities, that result in the loss of function of tumor-suppressor genes and/or gain of function or hyperactivation of oncogenes [1, 2]. More than $200 billion by one estimate have been invested in basic and clinical research to eradicate these malignant diseases since 1971, the year Richard Nixon declared a war on cancer [3]. Cancer still remains a leading cause of death in the world since most cancers remain incurable although mortality rates have declined in recent years owing to earlier detection and the availability of new treatment options [4].

The current anticancer armamentarium includes many active agents that are applied across cancer types. Most drugs have a small therapeutic index and barely discriminate between malignant and normal cells. It causes a problem that is further amplified by the almost inevitable onset of resistance and subsequent relapse [4]. In recent years, one focus
has shifted to target particular signaling pathways that drive inappropriate cell growth and survival, therefore offering a promise of greater specificity coupled with reduced systemic toxicity. Such strategy relies on the basic research to identify a particular target or a particular pathway undergoing mutation or deregulation, which is a valuable indicator for causing a specific cancer [4, 5]. Another focus is to search for more natural anticancer products and investigate their molecular mechanisms although the role of natural products in drug discovery has undergone many changes in the past 30 years, with a noticeable decline in participation by the major pharmaceutical companies by the mid-1990s [6, 7].

**Introduction to traditional Chinese medicine and its history**

Traditional Chinese medicine comprises medicinal products, often multiple combinations of compounds, primarily from plants, and also from animals and minerals to treat and relieve the symptoms of many different human diseases. It is frequently regarded with some skepticism by Western academic medicine since Western medicine currently employs pure, single compounds, either natural or synthetic with their detailed molecular mechanisms and metabolisms. However, prominent examples of isolated therapeutics derived from Chinese plants are established in modern medicine without being treated with the same reluctance as traditional herbal products. Among them are the ion channel blocker tetrandrine from *Stephania tetrandra*, the CNS stimulator ephedrine from *Ephedra sinica*, the anti-malarial artemisinin from *Artemisia annua*, inhibition of the TNF-alpha in human oral cancer cells through the NF-kappa B, AKT, and ERK-dependent pathways by Gan-Lu-Yin (GLY), a traditional Chinese herbal formula [8], and the well-known anticancer agents camptothecin from *Camptotheca acuminata* or paclitaxel from *Taxus chinensis* [9].

Traditional Chinese medicine was firmly established by 200 BC, and a listing of medicinal herbs and herbal formulations had been developed by the first century AD. Now, over 4,500 herbs, 300 mineral and animal extracts, and over 400 formulas are used in China. Herbal formulations may consist of 4 to 12 herbs, 300 mineral and animal extracts, and over 400 formulas had been developed by the first century AD. Now, over 4,500 BC., and a listing of medicinal herbs and herbal formulations traditional Chinese medicine was firmly established by 200 AD. The National Center for Complementary and Alternative Medicine, announced in 2005 that it has funded five new research centers on Chinese herbal medicine in cancer treatment.

**General application and limitations of traditional Chinese medicine in cancer treatment**

It is suggested that herbal medicine can be used along with conventional treatment prescribed by oncologists, such as radiation therapy and chemotherapy, to ease the side effects of conventional cancer therapies, control pain, improve quality of life, strengthen the immune system, and in some cases, stop tumor growth and spread. On the other side, scientific evidence of bioactive chemical components, efficacy and safety is frequently missing, and quality management needs to be improved. More research using modern analytical and chemical techniques is needed to determine the effectiveness of these individual substances [13], to ensure efficacy and safety, to provide qualitative and quantitative analyses for their molecular targets and metabolism, and to develop new, effective and safe world-class drugs [14]. Another challenge is how to verify traditional Chinese medicine therapies in the modern clinical setting. Most Chinese herbal preparations are a combination of many natural products, and the active compounds. They can vary drastically depending on where they were grown or collected, or even from one year to the next in the same area. Because of this natural variability, scientists stress the need for rigorous quality control in production and a thorough understanding of the molecular mechanisms behind the efficacy observed in any Chinese herbal preparation [9]. There is evidence from randomized clinical trials that some Chinese herbs may contribute to longer survival rates, reduction of side effects, and lower risk of recurrence for some cancers, especially when combined with conventional treatment [15]. Many of these studies, however, are published in Chinese, and some of them do not list the specific herbs that were tested. Some of these journal articles do not describe how the studies were conducted completely enough to determine whether they use methods comparable to those used in Western clinical research.

In summary, researchers’ interest in natural compounds has been raised based on the urgent need to develop new drugs with improved features for cancer therapy. The isolation of known Chinese herbs with anticancer activity and the elucidation of their chemical structures enable pharmacological and molecular biological investigations comparable to chemically synthesized compounds. Identification of molecular targets of natural products and related signal transduction pathways will allow the clarification of modes of action and design of novel
anticancer drugs. As a result, the review introduces traditional medicine to the Western countries and bridges the gaps between Western and eastern medicines.

Discussions on the Chinese medicinal herbs or other natural resources that have been evaluated to have anticancer activities

Introduction to the medicinal herbs that have been used in cancer treatment

In this review, we first discuss the Chinese medicinal herbs, which have been evaluated to have their anti-proliferative activity on cancer cell lines or tumors based on the literature. Many kinds of Chinese herbs have been confirmed to have the character of antitumor or anticancer by scientific reports in recent years. However, most of the reports have been focused on the clinical treatment of effectiveness for Chinese herbs. On the other hand, reviews about Chinese herbal related molecules on cancer cell apoptosis are seldom. In fact, Chinese medicine provides a rich pool of novel and efficacious agents for treating a variety of diseases, especially those that could not be cured by Western medicine. Thus interest in the use of traditional Chinese medicines for cancer prevention and treatment is increasing [16]. According to the published literature we have read, we discuss ten Chinese herbs: *Artemisia argyi*, *Artemisia annua* L, *Commiphora myrrha*, *Duchesnea indica*, *Ligustrum lucidum*, *Rheum palmatum*, *Salvia chinensis*, *Semen coicis*, *Scutellaria barbata* and *Vaccaria segetalis*. All of them have been described in the traditional literature of China, and reported to have more or less anti-proliferative activity by the research articles, however, the bioactive compounds and their efficacy of these plants have not yet clearly been scientifically documented [17, 18]. Many scientists even could not believe such kinds of descriptions about antitumor or anticancer effects of Chinese herbs because of lack of chemistry evidence of bioactive molecules in these herbs.

Two categories of the medicinal herbs in cancer treatment: anticancer activities and maintaining body balance

In general, the medicinal herbs used in cancer treatment can be divided into two categories although these two categories are not completely separated in vivo since they are related. One is that herb extracts have direct anticancer activity while the other is that herb extracts maintain human body balanced to bolster immune system for fighting diseases in combined with conventional tumor treatment strategies. Immune cells often constitute immunosuppressive microenvironment around tumors and alleviate tumor immune escape and tumorigenesis. *Artemisia argyi*, *Artemisia annua* L, *Commiphora myrrha*, *Duchesnea indica*, *Rheum palmatum*, *Salvia chinensis*, *Scutellaria barbata* and *Vaccaria segetalis* appear to contain the compounds with anticancer activity. In contrast, *Ligustrum lucidum* consists of the compounds, which can improve human immune system. *Semen coicis* may contain the compounds with anticancer activity and with immune system regulation activity as well. The general background on these herbs is briefly summarized in the following two paragraphs.

*Artemisia herbes* are one of the most popular plant species in Chinese traditional preparations and are frequently used as a common traditional Chinese medicine for the treatment of diseases such as malaria, anaphylaxis, hepatitis, cancer, inflammation, and infections by fungi, bacteria, and viruses. *Artemisia argyi* and *Artemisia annua* L have been reported to have the cytotoxic activity against some of cancer cell lines [19]. Artemisinin, isolated from *Artemisia herbes*, and its derivatives dihydroartemisinin, artemether and artesunate, have the potent anticancer activity in various cancer cells including those of leukemia and other cancer cell lines of breast, ovary, liver, lung, pancreas and colon [20-22]. The historical information regarding the use of *Commiphora myrrha* dates back centuries for treatment of infection, pain, swelling, leprosy and halitosis. The studies showed that it induces apoptosis in lung, pancreas, breast and prostate cancer cell lines in vitro and induces tumoricidal effects in mice [23]; *Duchesnea indica* has been documented as anti-inflammatory, astringent and anticancer folk medicines in the Chinese ancient medical works. It is often used for cancer therapy alone or as a main ingredient in the formulas with traditional reputed benefits for the treatment of cancer in China and in Japan. The aqueous extract of *Duchesnea indica* was reported to have anti-proliferative activity in vitro against many different types of cancer cells and showed anti-neoplastic activity in vivo [24]; *Rheum palmatum* L is traditionally applied to cancer therapy in Chinese medicine. Emodin (1,3,8-trihydroxy-6-methyl-anthraquinone), an active component contained in *Rheum palmatum* L, was found to have antitumor and antibacterial effects. However, with the exception of emodin, other anti-proliferative constituents of *R. palmatum* and their biological effects remain unclear [25]; *Salvia chinensis* is an herbal medicinal plant distributed in the southern part of the Yangtze River in China. It has been used as a Chinese folk medicine for the treatment of hepatitis, nephritis, dysmenorrhea and several kinds of cancers. The investigations showed that the chemical constituents of *Salvia* species were rich in triterpenoids, diterpenoids, monoterpenes and polyphenolics, which have been demonstrated to have anti-proliferative effects in some human leukemia cell lines [26]; Kanglaite Injection (KLT) is a novel type anticancer injection prepared by extracting its efficacious anticancer component from *Semen Coicis* with the world advanced technology and formulated into a lipid emulsion for intravenous as well as intra-arterial injection. Currently, KLT has been extensively used in more than 2,000 hospitals in China, and the food and drug administration (FDA) of the United States has approved a phase II trial of KLT to test its efficacy in treating non-small-cell lung cancer. Experiences obtained from clinical applications have demonstrated that KLT is markedly effective in treating a variety of malignant tumors such as carcinomas.
Many of the botanicals, such as shiitake, licorice, and side effects are either not present or substantially reduced. This way the body’s natural balance is preserved, and the body’s own defense mechanisms rather than substituting for them. In contrast, traditional medical practitioners use botanicals as a remedy for various cancers, inflammation and urinary diseases in Chinese medicine. Its anti-inflammatory effect is confirmed to be the bioactive phytochemical flavones: Wogonin, Baicalein and Baicalin [28]. Recent studies have demonstrated that Scutellaria barbata extract has anti-proliferative properties towards leukemia cells probably via the mitochondrial signaling pathway [29]; Vaccaria segetalis is widely distributed in China and the seeds of this plant are used for promoting diuresis, activating blood circulation in traditional Chinese folk medicine. It has been reported that Vaccaria segetalis consists of saponins, plant glycosides, that have diverse functions including foaming and pore forming properties, as well as favorable anti-tumorigenic effects due to their diverse structures [30, 31].

Some of the Chinese herbs might not have direct anticancer activity, instead, they function in maintaining immune system balanced. Western medical doctors often seek to bolster immune system activity by providing either chemotherapeutic agent that arrest aberrant cellular growth, or bioengineered molecules similar to elements of our own immune complex. In contrast, traditional medical practitioners use botanicals and other natural substances to stimulate or to potentiate the body’s own defense mechanisms rather than substituting for them. This way the body’s natural balance is preserved, and side effects are either not present or substantially reduced. Many of the botanicals, such as shiitake, maitake, licorice, echinacea, ligustrum and astragalus, owe their effects to a group of polysaccharides, which play a vital role in the body’s immune defenses. Ligustrum lucidum fruit has been used for several centuries in Chinese medicine. Substantial empirical evidence indicates that ligustrum possesses immune-modulating effects, including cancer inhibition [32]. Today, ligustrum is an important herb for immune-system restoration after chemotherapy, as are astragalus and shiitake.

In summary, screening of extracts of medicinal plants for cytotoxicity on cancer cell lines is still an active area of research throughout the world, and it is entirely possible that novel anticancer agents, including those amenable to a semi-synthetic approach, will be discovered in the future. Plants are used in traditional Chinese medicine to treat diseases including cancer, indicating that there might still be value in drug discovery [33, 34].

Discussions on the bioactive compounds extracted from Chinese medicinal herbs

Importance in identifying bioactive compounds from Chinese herbs

Cancer chemotherapy has now produced cures in at least some categories of human cancers. There are still urgent needs for more potent, specific, and low toxic anticancer drugs. Small molecules derived from medicinal plant and microbial sources have long had a significant role in cancer therapy. As discussed earlier, some compounds have the biological function in improving body immune system to fight for diseases although they do not have direct anticancer activity. However, this type of compounds can still be used in tumor therapy in combination with conventional tumor treatment strategies to achieve synergistic effects with particular targeted tumor therapies. For example, PHY906 (KD018), an adjuvant based on a 1800-year-old Chinese medicine, enhanced the antitumor activity of Sorafenib by changing the tumor microenvironment [35]. As a class, these molecules have the advantage of having greater chemical diversity over typical synthetic chemical libraries. They also tend to have greater chemical complexity, often with complex stereochemistry. It is these features that make natural products very specific for particular targets, and it is these features that combinatorial libraries of relatively simple synthetic compounds try to mimic [33].

The challenges in identifying bioactive compounds in Chinese herbs and the solutions

It should be pointed out earlier there are many challenges in identifying real bioactive compounds from Chinese herbs for anticancer treatment. The first challenge is that most remedies in traditional Chinese medicine, as it turns out, are compound formulae. It means that one formula contains as many as 50 species of herbs and thousands of chemicals therein. It is not easy to identify active ingredients with biological significance from too many-compound mixtures. To tap into the deeper well of traditional Chinese treatments, researchers think they may need to look at how the mixtures of ingredients act in concert. It appears no easy way to solve this problem. Pharmaceutical companies, which are interested in traditional Chinese medicines over the past decades, have been using the characteristically Western approach. Scientists isolate bioactive ingredients and test them one at a time. The second challenge is that complex natural products are normally difficult, if not impossible, to produce on a reasonable scale for chemical synthesis and biological test. One way around this problem is to use understanding of the chemistry of the complex natural product to complete its synthesis from a more easily obtained naturally occurring precursor; this ‘semi-synthesis’ is the approach used to manufacture the anticancer agent paclitaxel, starting with 10-deacetylbaccatin, which is readily obtained from needles of the English Yew. This is a renewable source,
in contrast to natural paclitaxel, which is found in the bark of the rarer Pacific Yew tree [33]. Another way is to use organic synthesis approach to synthesize promising compounds. The third challenge is about compound specificity. It is possible that bioactive compounds identified from Chinese herbs have tumoricidal effects through non-cancer target mechanisms, spreading the effects throughout the whole body. In another word, it means that the isolated bioactive compounds could have pronounced cell-killing activity on target cancer cells and on non-target normal cells as well. The only way to solve this problem is to identify their molecular targets, and then decide whether it is possible to modify the compounds to increase their molecular target specificity or to develop a drug delivery system specifically to tumors or leukemia cells. Although there are challenges, a few of the potential bioactive compounds including Pseudolaric acid A and B, Emodin, Genistein, and Triptolide have been identified from natural medicinal herbs.

The potential bioactive compounds identified from the Chinese herbs

Pseudolaric acids and their analogs

Pseudolaric acid A and B (Figure 1), with a unique scaffold, were initially isolated as the major compound (0.5–0.8% in the bark) from the root and trunk bark of *Pseudolarix kaempferi* Gordon (Pinaceae), which is a well-known traditional Chinese medicine [36]. Pseudolaric acids and their analogs exhibited significant cytotoxic activities against numerous tumor cell lines [36, 37] and strong antifungal activity [38]. Due to the unique structural scaffold and the significant anticancer activity, pseudolaric acids or their analogs have the potential value as an anticancer drug or a drug lead. Therefore, it has been an attractive structure for synthetic chemists [39]. Pseudolaric acids were reported to induce apoptosis of cancer cells through Bax/Bcl-2 pathways by elevating the level of Bax expression and down-regulating Bcl-2, and lead to the arrest of cancer cells at the G2/M phase of the cell cycle [40, 41]. However, it is not clear what are the molecular targets of pseudolaric acids related to their anticancer activity. One of potential molecular targets of pseudolaric acids is microtubulin, a polymer of α tubulin and β tubulin heterodimers, which is indispensable to the mitotic process. Thus, microtubules have been suggested as one of best validated targets known today [42, 43]. A number of compounds with diverse chemical structures interact with tubulin. Well known examples include the vinblastine and the paclitaxel classes of compounds. Although some of these microtubule-targeting agents, such as paclitaxel, originally isolated from *Taxus brevifolia* and the most active anticancer drug used in the clinic [9], have proven to be very effective in a range of malignancies, not all tumors are equally susceptible to these agents [43]. Furthermore, neurotoxicity and acquired drug resistance are common problems associated with the use of tubulin inhibitors [43]. In addition, recent observations indicate that microtubule-targeting agents exhibit antiangiogenic or antivascular properties [41]. This prompts the efforts to optimize the specificity and activity of known microtubule-targeting drugs as well as the development of novel anticancer drugs.

**Figure 1.** The chemical structures of pseudolaric acid A (top) and B (bottom)

Emodin:

Anthraquinones represent a large family of compounds having diverse biological properties. Emodin, 1,3,8-trihydroxy-6-methylanthraquinone (Figure 2), is a naturally occurring anthraquinone present in the roots and barks of numerous plants, molds, and lichens, and an active ingredient of various Chinese herbs [44]. Emodin, first assigned to be a specific inhibitor of the protein tyrosine kinase p65/ck [45], has now reported to be the inhibitor of protein kinase casein kinase-2 (CK2) [46, 47]. The activity of CK2 is elevated in a wide variety of tumors while its down-regulation can lead to apoptosis [47]. Additionally, because of its quinone structure, emodin may interfere with electron transport process and in altering cellular redox status, which may account for its cytotoxic properties in different systems [44]. At present, its role in combination chemotherapy with standard drugs to reduce toxicity and to enhance efficacy is pursued vigorously since emodin is a sensible candidate as a specific blocker of tumor-associated events. Identification of apoptosis as a mechanism of elimination of cells treated with cytotoxic agents initiated new studies deciphering the mechanism of apoptosis induced by emodin [44]. Emodin offers a broad therapeutic window, however, its target specificity and affinity need to be further investigated in order to become a member of anticancer armamentarium in future.

**Figure 2.** The chemical structure of emodin

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Genistein:
It belongs to isoflavone, a subclass of flavonoids [48]. Isoflavones are attracting considerable interest with regard to their anticancer properties as confirmed by in vivo [49, 50] and cell studies [51, 52]. Genistein, 5,7,4'-trihydroxyisoflavones (Figure 3), is one of the predominant compounds of soy isoflavones, which are natural chemoprotectors against cancer and are not toxic for normal cells [53]. Epidemiological studies have shown that, in Asia, the decreased occurrence of cancers, including prostate cancer, is associated with consumption of soy [54]. Genistein inhibits cell growth in several types of cancer, including prostate [55-57], breast [58, 59], lung [60], bladder [61, 62], and liver [63, 64], and inhibits angiogenesis in tumors [65]. It has been reported that genistein is poison of human topoisomerase II [66-68].

Triptolide:
It is a diterpenoid originally isolated from Tripterygium wilfordii Hook. f., a perennial vine growing in southern China [69], which has been used for centuries in traditional Chinese medicine for the remarkable efficient treatment of rheumatoid arthritis probably due to its potent anti-inflammatory and immunosuppressive activity [34, 70-72]. Triptolide (Figure 4) is a major active component of Tripterygium root extracts and has potent anti-inflammatory and immunosuppressive activities [69, 73, 74]. In addition to these activities, triptolide also exhibits a strong anti-proliferative, and anticancer effect [75-78] sensitizes solid tumor cells [79] through transcriptional arrest and/or caspase-dependent cell apoptosis [80-83]. The hydroxyl group of triptolide has been demonstrated to play an essential role in the anticancer and anti-inflammatory effects although the exact molecular targets still remain unclear. Thus, an understanding of the structural basis and molecular targets for the therapeutic activity will not only unravel the molecular cascades involved in the therapeutic effects but also facilitate the development of effective but less toxic derivatives for the treatment of both cancer and inflammatory diseases [83].

Identification of in vivo targets of bioactive compounds extracted from Chinese medicinal herbs

Importance of identification of in vivo targets of bioactive compounds

In the past, anticancer drugs were identified and developed without focusing on a particular macromolecular target. Currently, the fields of molecular biochemistry, molecular biology, genetics and pharmacology, among other disciplines, have grown considerably in their ability to identify molecular targets. Remarkable progress in these disciplines has led to the identification of numerous proteins with key roles in the function of both normal and abnormal cells. It has allowed the formation of specific hypotheses about how modulating the function of defined proteins that are linked to disease could be a route to new drugs. Such disease linked proteins are commonly referred to as molecular targets [84]. Scientists are now searching for specific targets to treat cancers and with another aim of decreasing side effects. These targets exist in different cellular compartments (membrane, cytoplasm, nucleus) as proteins, nucleic acids, etc. The recent reviews describe the literature on the application of genomics, metabolomics and metabonomics and proteomics [85, 86] to elucidate the molecular mechanism of action of traditional Chinese medicines on various diseases including cancers. The identification of a good target for a particular type of cancer is critical because it will help to achieve a deeper understanding of the molecular basis of drug action at defined target(s) in the context of all the pathways and signals within and between cells [33].

Evaluation of computational approaches for identifying in vivo targets of bioactive compounds

Methods to identify biological targets of a drug lead:

The transformation of a lead molecule into a drug is a key step in the process of drug discovery, requiring the application of knowledge of the compound's absorption, distribution, metabolism and excretion (ADME) profile to optimize its ‘druga-
Most compounds that fail to reach the clinic, even though they might be high-affinity in vitro inhibitors of the desired target, often do so because of insufficient attention to these issues. In addition, even the most sophisticated and effective targeted therapy is likely to be subject to similar underlying clinical limitations as classic cytotoxic-based agents, such as resistance, and these, in turn will require ever-more cunning chemistry-based improvement. Therefore, there is now widespread agreement that drug-like and ADME properties should be incorporated as early as possible in the discovery cycle, rather than ’retro-fitted’, after the optimization of molecular recognition features [33]. It is striking that few of the anticancer drugs currently in the clinic have optimized ADME features.

There are two ways to identify biological targets of a drug lead. The dominant technique for target identification of new lead compounds in drug discovery is the physical screening of large libraries of biological targets against a chemical or the physical screening of large libraries of chemicals against a biological target (high-throughput screening). However, this experimental approach is time-consuming and expensive. An alternative approach, known as virtual drug-based screening, is to computationally screen large libraries of biological targets, which the potential drug binds to, and experimentally test those that are predicted to bind well. Such drug-based virtual screening faces several fundamental challenges, including sampling the various conformations of flexible molecules and calculating absolute binding energies in an aqueous environment. Nevertheless, the field has recently had important successes: new drugs have been predicted along with their target-bound structures significantly greater than with high-throughput screening. Since false-positive and false-negative predictions can be tolerant for the time being, a virtual screening still offers a practical route in the early stage of discoveries of new reagents and leads for pharmaceutical research. The best way is to use a combination of experimental and theoretical approaches.

**Importance and limitations of in silico screening in drug development**

There seems little doubt that the use of in silico screening is set to increase in the future as more macromolecular structures become available and computer power continues to increase [33]. Computational methodologies have become a crucial component of many drug discovery programs, from hit identification to lead optimization and beyond, and approaches such as ligand or protein structure based virtual screening techniques are widely used in many discovery efforts. Some of these computational methods include quantum mechanics (ab initio and density functional theories) and molecular mechanics (docking, molecular dynamics, and protein folding). Docking and molecular dynamics (MD) are the most commonly used computational tools for elucidating cancer targets. Using these tools, one can identify the recognition processes between ligands and targets at the atomic level. In addition, one can identify affinity and conformational changes of these molecular complexes [84]. Docking of small molecules to protein binding sites, one key methodology, was pioneered during the early 1980s, and remains a highly active area of research [87, 88]. When only the structure of a target and its active or binding site is available, high-throughput docking is primarily used as a hit identification tool. However, similar calculations are often also used later on during lead optimization, when modifications to known active structures can quickly be tested in computer models before compound synthesis. The docking process involves the prediction of ligand conformation and orientation (or posing) within a targeted binding site. In general, there are two aims of docking studies: accurate structural modeling and correct prediction of activity [88]. In addition, a purely computational chemistry approach is increasingly used to counter the problem that even libraries containing very large numbers of compounds do not have enough chemical diversity to represent more than a small fraction of chemical types. This is in silico screening of virtual chemical libraries. With the marked increase in computer power during the past decade, it has become increasingly feasible to develop so-called in silico approach to drug discovery whereby large virtual libraries of compounds can be screened against the known structure of a protein or vice versa. In conclusion, use of such computational tools to identify biological targets of anticancer drugs from natural medicinal herbs in combined with chemical analysis, organic synthesis and chromatography is promising.

The computational-intensive procedures have several practical problems. First, they are not able to account adequately for large-scale active-site flexibility, and even restricted flexibility carries significant computational penalties. Second, the scoring functions are best able to cope with large differences between ligands rather than small, subtle ones, and cannot reliably calculate absolute binding affinities. The results from the theoretical calculations need to be compared with experimental data.

Computer-assisted drug design has supported pharmaceutical research and development for over three decades. An increasing number of studies have reported computations of the standard (absolute) binding free energy of drugs to proteins using MD simulations and explicit solvent molecules that are in good agreement with experiments. This encouraging progress suggests that physics-based approaches hold the promise of making important contributions to the process of drug discovery and optimization in the near future. The common approaches in MD simulations contain restraining potentials, which may be activated and released during the simulation for sampling efficiently the changes in translational, rotational, and conformational freedom of the drug and protein upon binding. However, such restraining potentials add bias to the simulations.
The solution for that is to rigorously remove these effects to yield a binding free energy that is properly unbiased with respect to the standard state [89].

An example of in vivo molecular targets of bioactive compounds: camptothecin-derived anticancer agents

Molecular target(s) of camptothecin-derived anticancer agents: topoisomerases

An ideal anticancer drug must be selective and cytotoxic only to cancer cells. The standard chemotherapy inhibits vital molecular targets such as topoisomerase, microtubule, DNA synthesis since common cancers have no specific targets [90]. The topoisomerases have essential roles in the key cellular process of DNA replication. The mammalian genome encodes seven topoisomerase genes: four that encode type I topoisomerases and three that encode type II topoisomerases (TOP2α and TOP2β and SPO11). The four mammalian type I topoisomerase genes include nuclear topoisomerase I (generally abbreviated TOP1), the mitochondrial topoisomerase I (TOP1MT) gene and two genes that encode TOP3α and TOP3β [91]. Elevated topoisomerase I levels in tumors are a factor in the antitumor activity of topoisomerase I inhibitors [92]. Camptothecin, a cytotoxic pentacyclic alkaloid, which was isolated from the bark and stem of Camptotheca acuminata, a tree native in China, inhibits the DNA enzyme topoisomerase I. Camptothecin and its derivatives, for example, topotecan and irinotecan are presently the only topoisomerase I inhibitors approved for cancer treatment since they target only topoisomerase I with high specificity [91, 93]. Topotecan is prescribed for the treatment of ovarian and lung cancers whereas irinotecan is for the treatment of colorectal cancers [94]. Topoisomerase I-targeting drugs and the topoisomerase II-targeting drugs doxorubicin, amsacrine, etoposide and teniposide, stabilize the covalent topoisomerase–DNA complex, thereby preventing DNA religation. In the presence of inhibitors, topoisomerase I is down regulated and targeted to the ubiquitin/proteasome pathway [92] and ultimately cause cell death during the S-phase of the cell cycle [94] (Figure 5).

Drawbacks of camptothecin-derived anticancer agents

Despite clinical success due to their pharmacological unique, there are several problems with camptothecin-derived anticancer agents. A major limitation is from the chemical equilibrium between camptothecin lactone form and the E-ring-opened form. The E-ring opened carboxylate form has less than 10% the potency of the lactone form as a topoisomerase I inhibitor and is inactive in cell culture [94, 95] (Figure 6), perhaps due to inability to cross the cell membrane [92, 96]. However, this argument appears to be controversial since Staker and co-workers reported the X-ray structure crystal structure of the ternary complex containing human topoisomerase I covalently joined to a DNA duplex and bound to carboxylate form of topotecan [97]. Pommier also speculated that when the camptothecin lactone enters the topoisomerase I-DNA active site, E-ring opening is activated [98]. Camptothecin analogs suffer from another drawback which further limits antitumor efficacy [92]. Although these drugs can freely enter cells via passive diffusion across cell membranes, their intracellular concentration is greatly reduced by efflux pumps in a wide variety of tissues. Multi-drug resistance (MDR) results from drug efflux by the well characterized P-glycoprotein (P-gp) [99]. Both topotecan and irinotecan are substrates for P-gp [92]. Additionally, camptothecins produce side effects (such as leukopenia) that limit the dose that can be safely administered and, therefore, antitumor efficacy. The diarrhoea that is induced by irinotecan can be severe and is probably due to ‘off-target’ effects that are related to the bis-piperidine that confers water-solubility [91]. Topoisomerase I cleavage complexes need to be maintained long enough to be converted into DNA damage. However, camptothecins rapidly diffuse from the complexes, which means that they must be given as a prolonged infusion to maintain persistent cleavage complexes [91].

Efforts to chemically modify camptothecin-derived anticancer agents to improve its efficiency and specificity

It is clear that topoisomerase I remains a target of active interest in the development of new anticancer agents because topoisomerase I inhibitors are clearly active and effective anticancer drugs and also because these current inhibitors are molecules that can be improved upon. Investigation of to-
poisomerase-DNA-drug complexes may lead to new drugs that improve therapeutic benefit to patients. Two modifications of the camptothecin E-ring have been introduced to alleviate the problem. One way to stabilize the crucial E-ring is to insert a methylene group between the keto-group and the hydroxyl-substituent. The homocamptothecins, such as diflomotecan (BN80915 is in early clinical trials), are this type of modifications, which conserve or enhance topoisomerase I enzyme inhibition. In contrast to camptothecins, homocamptothecins undergo slow lactone ring opening in plasma [92, 100, 101]. Another way to stabilize the E-ring is to remove the lactone group, which completely blocks E-ring opening, as in the keto derivatives that retain high anti-topoisomerase I activity. The cyclobutane methylenedioxy derivative, S39625, is this type of modification [91]. In addition, the efforts have been made to prolong the lifetime of the topoisomerase I-DNA-drug ternary complex, which directly mediates their cytotoxocities [102]. Therefore, designing topoisomerase I inhibitors that have more stable E-ring and greater binding persistence represents an attractive approach for achieving more efficacious anticancer agents [103, 104].

The current synthetic and semi-synthetic strategies and studies of camptothecin action mechanisms have facilitated the development of camptothecin analogs with improved properties including lactone stabilization, solubility and drug transport mechanisms, tumor cell recognition and enhancement of DNA sequence specificity [95]. Majority of these analogs are substitutions, additions and deletions of A/B rings, C and D rings and E-ring of the pentacyclic structures. It is generally suggested that either replacement or substitution at C and D rings would loss the activity except 14-azacamptothecin, which exhibited reasonable potency as a topoisomerase I poison [105]. Most structural model studies indicated that the modifications at positions C-7, C-9, C-10 and sometimes C-11 of A and B rings have the possibility to enhance potency while camptothecins do not tolerate modifications at position C-12. Some modifications result in superior or equivalent topoisomerase I inhibitory and antitumor activity in vitro, which include lurtotecan, exatecan, compound 67, S39625, ST1976 [95, 106-108] (Figure 7). E-ring plays a key role in supporting both efficient topoisomerase I inhibition and in vivo potency. The replacement of C-21 by N and S, and replacement of 20-OH with NH₂, H and halogens reduce the tendency of E-ring to open. However, these compounds are either essentially inactive or have significantly diminished activities [96, 102]. In contrast, a few of E-ring modifications including homocamptothecin, 10,11-difluorohomocamptothecin, compound 85 (Figure 8), not only exert impressed potency and stability of lactone, but also exhibit strong anti-proliferative activity against numerous cell lines [95, 109-112]. However, the mechanistic basis of camptothecin analogs is not well understood. Correspondingly, it is not possible to predict the binding properties.

In-silico modeling studies to improve camptothecin-derived anticancer agents

As stated earlier, MD is a well-established method for investigation of structural and dynamics properties of proteins and nucleic acids [113]. The molecular forces between atoms and molecules are now sufficiently well characterized to enable calculation (and prediction) of thermodynamic quantities and to follow trajectory of interacting molecules in a given system as a function of time. The trajectory simulations yield a molecular level view of the system, which may not be accessible by experiment. These tools are increasingly being employed to gain molecular insights, rationalize experimental data, and predict drug-protein interactions. This in-silico modeling has the significance and the need in the current drug development. Previous MD simulations successfully revealed the dynamics properties of topoisomerase I [114, 115] and persistence of the various camptothecin analog-ternary complexes [104]. The MD study indicated a direct communication between human topoisomerase I Thr718Ala mutation site and regions located relatively far away, such as the linker domain, that with their altered flexibility confer a reduced DNA relaxation efficiency. These results provide evidence that the comprehension of the topoisomerase I dynamical properties are an important ele-
ment in the understanding of its complex catalytic cycle [115]. Another MD study revealed that the most persistent camptothecin and its analogs (i) have higher calculated free-energy barriers for drug dissociation from the flanking base pairs, (ii) are less sensitive to changes in the rotation angles of the flanking base pairs, (iii) form stronger van der Waals and hydrophobic interactions, and (iv) have larger stacking areas with the flanking base pairs. Collectively, their study demonstrates that MD simulations can be used to gain mechanistic insight into the molecular basis for the persistence of the ternary complexes and predict the persistence of such complexes during the drug discovery process [104]. It will be interesting to take the advantages of this in-silico modeling to study the binding properties of topoisomerase l-targeting drugs lurtotecan, exatecan, compound 67, S39625, ST1976 homocamptothecin, 10,11-di-fluorohomcamptothecin, compound 85 [95, 106-112] to gain insight into their molecular basis.

Future directions of anticancer drugs including camptothecins

It is clear that camptothecins are cytotoxic to tumors and leukemic cells. However, camptothecins are cytotoxic to normal proliferating cells, for example, bone marrow cells, which can result in anemia and/or problems in immune system and further foster the development of drug resistance. It seems that all the cancer drugs have this problem except imatinib, a specific Bcr-Abl-leukemia cell inhibitor, for example, microtubule-targeting drug paclitaxel [43], cyclin-dependent kinase-targeting drug indirubin [116]. All these drugs have the cytotoxicity to normal proliferating cells. One of the solutions is to develop pro-camptothecin drugs, which should be stable in vivo and far less toxic than their parent forms, and activated specifically when they reach in or within the microenvironment of the tumor cells. Another solution is to identify novel prognostic factors at the molecular level. The idea is to predict drug resistance or severe side effects in individual tumors and patients. With this information, a treatment could be adjusted to the individual requirements of each tumor patient to obtain optimal treatment results with most effective tumor eradication and with tolerable side effect.

Concluding Remarks

Primary cause of a particular type of cancer is often due to mutation(s) of a particular gene. There exist anti-proliferative compounds in medicinal herbs. However, those compounds are often not molecular target-specific. Traditional Chinese medicine views the body as an energetic system in a dynamic balance, thus helps restore the body to balance and works on an energetic level to affect all aspects of a person: mind and/or body. Many Chinese medicines have been demonstrated to be effective in clinical practice. It is essential to identify bioactive compounds both singly and in combination – from active ingredients, active fractions, and active herbal formulations and subsequently to identify their in vivo targets. In conclusion, herb extracts could be used to maintain body balance for fighting diseases including cancers in combined with mechanism-based treatment strategies.

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