

Selected Secondary Plant Metabolites for Cancer Therapy

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ABSTRACT

Secondary plant metabolites reveal numerous biological activities making them attractive as resource for drug development of human diseases. As the majority of cancer drugs clinically established during the past half century is derived from nature, cancer researchers worldwide try to identify novel natural products as lead compounds for cancer therapy. Natural products are considered as promising cancer therapeutics, either as single agents or in combination protocols, to enhance the antitumor activity of additional therapeutic modalities. Most natural compounds exert pleiotropic effects and modulate various signal transduction pathways. A better understanding of the complex mechanisms of action of natural products is expected to open new perspectives in coming years for their use alone or in combination therapies in oncology. Two major strategies to identify novel drug candidates from nature are the bioactivity-guided fractionation of medicinal plant extracts to isolate cytotoxic chemicals and the identification of small molecules inhibiting specific targets in cancer cells. In the present review, we report on our own efforts to unravel the molecular modes of action of phytochemicals in cancer cells and focus on resveratrol, betulinic acid, artesunate, dicentrine and camptothecin derivatives.

Key words: Apoptosis, Bioactivity-guided fractionation, Drug development, Drug resistance, Epidermal growth factor receptor, Phytochemicals, Targeted tumor therapy

INTRODUCTION

In the western industrialized countries, medicinal plants are becoming increasingly interesting not only in the general public, which is interested in complementary and alternative medicine, but also in academic and industrial research, which is working on the isolation of novel plant compounds as lead compounds for the generation of pharmacologically improved active molecules^[1-8]. It is sometimes overlooked that phytotherapy is the oldest form of medical intervention. According to the World Health Organization (WHO) more than three-quarters of the world population use medicinal plants^[9]. Traditional folk medicine preparations are usually cheaper than synthetic drugs, which are frequently not affordable for many people in developing and emerging countries. The WHO has 21,000 plant species cataloged as medicinal plants in the world^[10].

The efficacy of medicinal plants is based on their chemical ingredients. This herbal medicine is basically accessible to scientific, hypothesis-driven research and can be deferred from mystic or esoteric approaches. Phytochemistry represents a discipline of pharmacy dealing with the chemical constituents of plants, which can be studied by classical pharmacological, molecular biological and medical methods for their efficacy, *e.g.* in cell culture and animal experiments as well as in clinical trials.

Basically, primary and secondary plant metabolites can be distinguished from each other (Figure 1). While primary metabolites (sugars, fats, amino acids, etc.) serve for primarily nutrition and as starting materials for further biosynthesis (*e.g.*, starch, cellulose, etc.), secondary metabolites do not play

a role for diet. Rather, they are of crucial evolutionary importance for the biological “fitness” of plants. On the one hand, they are weapons in the fight against pathogens such as viruses, bacteria, and protozoans. Hence, they take over the defense role the immune system has in animals. On the other hand, they serve as fragrances or dyes to attract pollinators. The fact that certain plants have pharmacological properties in human beings seems to be nothing but an evolutionary byproduct of nature. However, one must not ignore the fact that many plants are extremely toxic and can be dangerous and even life-threatening to humans. The popular notion that “green medicine” is a fundamentally gentle medicine without potential side effects is misleading in this context.

Since the isolation of the first alkaloid, morphine, from the opium poppy (*Papaver somniferum*) in 1804, a lot of valuable secondary plant metabolites have been described, which have long been firmly established in pharmacology. About one third of all current medicines based on natural products. The fraction of natural substances is even considerably higher, when it comes to cancer therapy. More than two thirds of all cancer drugs are natural substances (plant substances, antibiotics), derivatives thereof, or drugs based on principles of action that have been taken from nature^[11]. In a sense, therapeutic antibodies used in recent years can also be understood as biological drugs. Known cytotoxic secondary plant substances and their semisynthetic derivatives established in clinical oncology are, for example, Vinca alkaloids (vincristine, vinblastine, vindesine, vinorelbine) from the Madagascar periwinkle (*Vinca rosea* resp. *Catharanthus roseus*), taxanes (paclitaxel, docetaxel) from the Caribbean

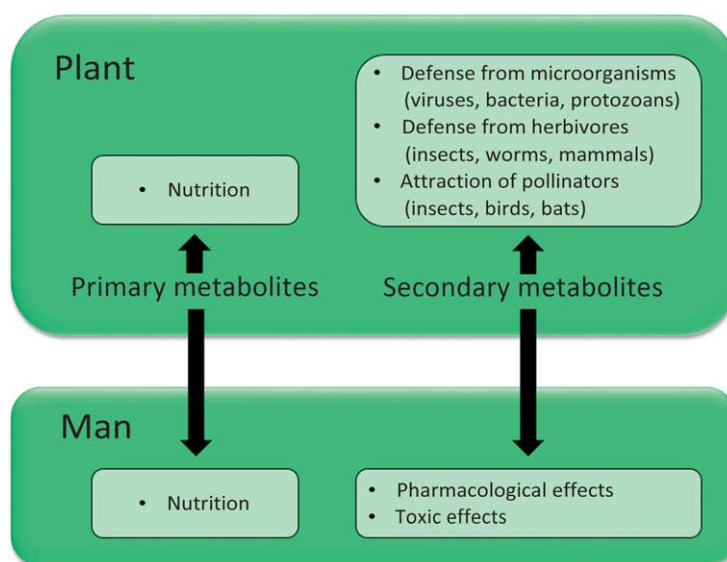


Figure 1. Functions of primary and secondary plant metabolites.

yew (*Taxus brevifolia*), epipodophyllotoxins as derivatives of podophyllotoxin from the American Mayapple (*Podophyllum peltatum*) and irinotecan and topotecan as derivatives of camptothecin from the Chinese lucky tree (*Camptotheca acuminata*). These examples of phytochemicals that made their way into clinical cancer therapy illustrate that secondary plant metabolites represent a valuable resource for drug development. This is all the more significant when considering the enormous chemodiversity of plants on this globe.

In addition to cancer therapy, secondary plant metabolites play an important role in chemoprevention, i.e. the prevention of carcinogenesis. It is therefore not surprising that research on herbal drugs is carried out worldwide with much enthusiasm in recent years. Plants have significant potential for cancer research, especially since only about 15% of all medicinal plants have been systematically investigated for their chemical constituents thus far.

Generally, two different concepts of research can be distinguished:

- (1) Bioactivity-guided isolation of natural products, in which active substances are isolated by successive purification steps and parallel activity testing. This is a classical method in phytochemistry.
- (2) Targeted tumor therapy, which seeks to address proteins in tumors. This is a more recent research approach that aims to reduce the side effects on normal tissues by drugs with higher tumor selectivity. In natural product research, this concept is innovative and not well explored.

In the present review, we provide some examples from our own research for both strategies. We will introduce first three representatives from different chemical classes: the polyphenolic stilbene resveratrol, the pentacyclic triterpene carboxylic acid, betulinic acid and the semisynthetic artesunate as a derivative of the sesquiterpene artemisinin. These three substances were identified by conventional screening methods from plants. Finally, we introduce possibilities for targeted therapy of

epidermal growth factor receptor (EGFR)-expressing tumors with the alkaloids, dicentrine and camptothecin derivatives.

RESVERATROL

Among the popular phytochemicals of recent years is resveratrol. This polyphenol is widely distributed in nature and is among other constituents, an important health-promoting component of red wine^[12]. Although resveratrol for years already in clinical trials for chemoprevention in the testing stage^[13], there is as yet few convincing data demonstrating the anti-tumor effect of resveratrol, particularly in advanced tumor stages. Not only as chemopreventive agent, but also as a cancer drug resveratrol could play an important role in the years to come. Preclinical and experimental studies have shown that resveratrol both inhibits tumor growth and promotes tumor cell death. The underlying molecular mechanisms are partly unraveled. Resveratrol influences a variety of intracellular signaling pathways that control tumor growth. On the one hand, resveratrol inhibits proliferation-promoting processes, such as PI3K/Akt/mTOR the signal path and the LF κ cascade B^[14–17]. On the other hand, resveratrol promotes the induction of cell death by suppressing apoptosis-inhibiting factors and inducing proapoptotic molecules^[18]. Moreover, studies on tumor cell cultures demonstrated that resveratrol can also be used to increase the effectiveness of chemotherapy. In the presence of resveratrol, a significantly higher rate of cell death was recorded in tumor cells incubated with various cytotoxic compared to tumor cell cultures that received only treatment with cytostatic agents^[19]. Moreover, resveratrol amplified the sensitivity of tumor cells to death receptor-induced apoptosis^[19–21]. These studies on the underlying mechanisms of action thus provide a rational basis for the introduction of resveratrol into existing treatment regimens in oncology. Due to the elucidation of the complex modes of action of resveratrol, new application perspectives are emerging for resveratrol as a single agent or in combination therapy in the prevention or treatment of cancer.

BETULINIC ACID

Betulinic acid, a pentacyclic triterpene, represents another biologically active natural product^[22]. As the name suggests, betulinic acid is mainly found in the birch bark (*Betula alba*). Betulinic acid was isolated already in the 18th century from plants and described as a natural product. Of the diverse biological properties of betulinic acid, the significant inhibition of tumor growth is mainly known^[23]. This antitumor activity of betulinic acid has been attributed to several mechanisms, wherein the induction of apoptosis plays an important role. Studies of tumor cell cultures and in cell-free systems showed that betulinic acid directly affects mitochondrial function and stimulates the release of mitochondrial proteins such as cytochrome c and Smac from the mitochondria into the cytoplasm, which ultimately leads to cell death^[24]. Of particular interest is the fact that betulinic acid significantly increases the effectiveness of chemotherapy or radiation in preclinical models. These results indicate that betulinic acid could strengthen the effectiveness of conventional cancer therapies. Interestingly, betulinic acid was active even in different models of primary or secondary drug resistance^[25, 26]. This could be due to the fact that the mechanism of action of betulinic acid differs from most conventional cancer drugs, so that generally no cross-resistance occurs. Thus, betulinic acid and its derivatives may open new perspectives to treat tumors that are resistant to conventional therapies.

ARTESUNATE

The anti-malarial drug artemisinin also shows potent activity against cancer cells. Starting from a screening program to analyze cytotoxic substances of medicinal herbs used in traditional Chinese medicine, the tumor cell killing effectiveness of artemisinin was recognized^[27]. We performed a bioactivity-guided screening more than 250 plant extracts regarding their cytotoxic properties. Active extracts were fractionated by chromatographic techniques and pure active substances were isolated, whose chemical structure has been elucidated by nuclear magnetic resonance (NMR)^[28]. Since artemisinin is poorly water soluble, further investigations were performed with the semisynthetic derivative artesunate. We found that artesunate was not only active against cell lines of different tumor types^[29, 30], but also against viruses, trypanosomes and even against plant gall tumors^[31, 32].

To elucidate the mechanisms of action of artesunate *in vitro* and *in vivo*, we used molecular and pharmacogenomic methods^[33–37]. Artesunate affects certain signal transduction pathways such as the anti-oxidative stress response^[38, 39] and the signal transmission of the epidermal growth factor receptor (EGFR)^[40]. Furthermore, artesunate inhibits the formation of nitric oxide (NO), inhibits the cell cycle regulators CDC25A and TCTP^[41] and induces oxidative DNA damage, which are repaired by an ATM/ATR-directed DNA double-strand break repair mechanism^[42, 43]. It is fortunate that its activity is not hampered by multidrug resistance-conferring ATP-binding cassette (ABC) transporters, indicating that otherwise drug-resistant tumors remain

still responsive to artesunate^[41]. Furthermore, it inhibits tumor angiogenesis^[35, 44]. Despite its multi-target specificity artesunate is a remarkably well-tolerated, with few side effects drug, which has been shown in meta-analyses with thousands of malaria patients^[45, 46].

Artesunate also inhibited the growth of human xenograft tumors in nude mice^[44]. Two successful therapeutic trials in patients with melanoma uveal melanoma (choroidal tumor) and a pilot study of 10 cervical cancer patients encouraged to carry out further controlled clinical studies^[47, 48].

TARGETED THERAPY WITH NATURAL PRODUCTS

The concept of targeted therapy with natural products from medicinal plants and subsequent analysis of underlying mechanisms of action was successful in the examples shown above. Using this strategy, substances with multiple mechanisms of action that attack various targets in tumor cells can be found. In recent times, we also attempted to find anti-cancer compounds, which targeted tumor proteins more specifically, in order to increase the tumor specificity of tumor therapy and to minimize the side effects on normal tissue. The prototype of tumor-targeted cancer therapy is imatinib (Gleevec®), a small molecule that binds to the Bcr-Abl fusion protein in chronic myeloid leukemia and gastrointestinal stromal tumor c-Kit protein. In recent years, a number of other targeted therapies have been introduced to the market. We dealt with the question of whether a targeted therapy with secondary plant metabolites is also possible and selected the epidermal growth factor receptor (EGFR) as a target molecule for drug screening.

As a starting point for these studies, a chemical database was developed with 2400 natural products from plants used in traditional Chinese medicine^[31]. Using bioinformatic methods (molecular docking) have been identified dicentrine and camptothecin derivatives as candidates, which bind *in silico* with high binding affinity to the tyrosine kinase domain of EGFR^[49]. The tyrosine kinase domain is the binding site for erlotinib, a clinically well-established small molecule inhibitor of EGFR. Our bioinformatical docking showed that dicentrine binds to the same binding pocket as erlotinib, but at other amino acids as erlotinib. This may become significant in cases, where tumors become resistant to erlotinib by the outgrowth of EGFR point-mutated cell subpopulations. New substances such as dicentrine may bind to other amino acids in the EGFR pharmacophore than erlotinib and thereby kill erlotinib-resistant tumor cells. In fact, we were able to show that the growth of erlotinib-resistant EGFR-transfected cells are preferentially inhibited by dicentrine compared to non-transfected control cells^[50]. To find out which EGFR downstream signaling pathways are involved in the inhibitory effect of dicentrine, we have generated transcriptome-wide microarray-based mRNA expression profiles of treated and untreated cells. Dicentrine activated BRCA1 mediated DNA damage response, p53-dependent signal transduction and G1/S and G2/M cell-cycle regulation^[50]. The activation of these pathways can be explained by the intercalation of dicentrine into DNA and the

induction of DNA strand breaks by the inhibition of DNA topoisomerases, which also leads to cell cycle arrest^[51].

Camptothecins are known as inhibitors of DNA topoisomerase I (TOP1), although the clinical correlation between TOP1 expression or enzyme activity and the response of tumors to camptothecin derivatives is surprisingly weak^[52]. This suggests that camptothecin could have other targets in addition to TOP1. Our bioinformatical investigations indicated that EGFR may be a therapeutic target for camptothecin derivatives. We found that camptothecin (CPT) and camptothecin 20-N,N-glycinate (CPTG) bind to the same pharmacophore on EGFR as erlotinib, but to some other amino acids^[50]. EGFR-transfected cells revealed preferential cytotoxicity of the two camptothecins compared to non-transfected control cells. Microarray experiments indicated that signaling pathways are activated by the substances that are responsible for the G2/M checkpoint regulation after DNA damage, the aryl hydrocarbon receptor signaling, the xenobiotic metabolism and endoplasmic reticulum stress^[50]. These results show that EGFR as target molecule in addition to TOP1 can be postulated for camptothecins and that these compounds exert multifactorial effect on cancer cells.

Erlotinib resistance might be overcome by combination treatments with established EGFR inhibitors and natural products. Therefore, we assumed that substances acting on different sites of the EGFR signaling cascade could be particularly suitable for this purpose. To test this hypothesis, we treated EGFR-transfected cells with erlotinib as established EGFR tyrosine kinase inhibitor in combination with artesunate, which is known to inhibit EGFR downstream kinases^[40]. In fact, isobologram analysis showed that this combination resulted in a synergistic growth inhibition^[53].

PERSPECTIVES

Studies in recent years have shown that secondary plant compounds have great potentials for use in cancer therapy. However, in many cases, little is known about the mechanisms of action involved. The further clarification of molecular mechanisms of action will promote the rational use of phytochemicals as anticancer drugs and could open up new perspectives in oncology.

CONFLICT OF INTEREST

There is no conflict of interest.

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