

Molecular Target-Guided Tumor Therapy with Natural Products Derived from Traditional Chinese Medicine

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Abstract: A tremendous interest exists in the Western world in Traditional Chinese Medicine (TCM) with rapidly increasing export rates of TCM products from China to Europe and USA. This led to a national decision of the Chinese government to implement a “Plan for the Modernization of Chinese Medicine”. Concerning the use of Chinese medicinal herbs, two major directions can be distinguished. One field is phytochemistry and pharmacognosy. Secondary metabolites isolated from Chinese plants can be easily subjected to pharmacological, molecular biological, and pharmacogenomic analyses using methods of modern cell and molecular biology as exemplified for camptothecin from *Camptotheca acuminata* in the present review. The second field of interest is phytomedicine. Standardized international quality guidelines help to improve quality, safety and efficacy of Chinese medicinal herbs. Sustainability of natural products from TCM can be reached by breeding high-yield varieties or by biotechnological approaches. In the long term, natural products from TCM can contribute to the development of molecular target-guided therapies and individualized treatment strategies.

Keywords: Phytochemistry, pharmacognosy, phytomedicine, biotechnology, pharmacogenomics.

INTRODUCTION

Traditional Chinese Medicine (TCM) comprises medicinal products from plants, animals and minerals, acupuncture, and other practices. In the present overview, we focus only on herbal medicine (*ben cao*). Herbal prescriptions consist of a varying number of different medicinal plants and are used as extracts, decoctions and teas.

TCM is frequently regarded with some scepticism by western academic medicine, because:

1. TCM represents a holistic approach pointing to the entire human body, while western science and medicine is focused on mechanisms. Rather than analysis of the patient in entirety, it is only the disease that is analyzed at the cellular, molecular, and pharmacological level.
2. Scientific evidence of efficacy and safety is frequently missing, and quality management needs to be improved. Faked TCM preparations on the market further weaken the reputation of TCM in the scientific community outside China.

On the other side, 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 (*Stephania tetrandra*), the CNS stimulator ephedrine (*Ephedra sinica*), the anti-malarial artemisinin (*Artemisia annua*), and the well-known anticancer agents camptothecin from *Camptotheca acuminata* or paclitaxel from *Taxus chinensis*.

Currently, there is a tremendous interest in the western world in TCM. The export of TCM products from China to Europe results in annual sales of US \$ 180 million (<http://www.china.org.cn/english/2004/Aug/103236.htm>). In 1995, the worldwide recognition of TCM led to a national

decision of the Chinese government to implement a “Plan for the Modernization of Chinese Medicine”.

The considerable attractiveness of TCM during the past years raised the interest of phytochemists and pharmaceutical biologists to investigate the pharmacological basis of TCM. Two major directions of Chinese herbal medicine can be distinguished:

Phytotherapy Or Phytomedicine

Treatments using entire plant drugs, aromatic essential oils and herbal or floral extracts, which are applied as herbal teas, through massage, packs or wraps; therapies by water and steam, or inhalation.

Phytochemistry

Chemistry of the secondary metabolites found in medicinal plants and isolated chemical entities. This field is easily compatible with molecular pharmacology, molecular biology and pharmacogenomics.

PHYTOTHERAPY

To establish international standards for TCM, a number of efforts are being undertaken:

- Strengthening research on the physiological and pharmacological activity of TCM remedies.
- Basic pharmacological research to establish a scientific platform on the activity of medicinal products.
- Toxicological screening to monitor toxic side effects.
- Controlled, randomized clinical trials to provide evidence-based practice of TCM.
- Support of international collaborations of universities and pharmaceutical companies with scientists and clinicians worldwide.
- Standardization of production of Chinese herbal prescriptions by international quality guidelines such

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as Good Sourcing Practice (GSP) to guarantee authentication of medicinal plants, Good Agricultural Practice (GAP), Good Laboratory Practice (GLP), Good Manufacturing Practice (GMP), and Good Clinical Trial Practice (GCTP).

Quality control has not only the task to ensure proper composition of herbal prescriptions but also to avoid contamination with mycotoxins, pesticides, heavy metals, or other chemical toxins. Furthermore, faked herbal prescriptions with adulteration of drugs from western medicine, e.g., with glucocorticoids, have to be banned [1]. Another goal to modernize TCM is the application of advanced biotechnology for production and quality control of Chinese herbal prescriptions.

PHYTOCHEMISTRY

There is an almost unmanageable diversity of secondary plant metabolites, most of which have yet to be studied. Only 15% of all plants have been thoroughly investigated for their pharmacological active constituents [2, 3]. The scientific investigation of TCM describes the transition from TCM to scientific Chinese medicine (SCM). The scientific basis of medicinal plants including those from TCM is being analyzed worldwide pointing to the relevance of this topic [4]. A comprehensive survey of natural products from TCM is beyond the scope of the present review. Therefore, the reader is referred to recent comprehensive reviews [5, 6]. We have recently performed a search for natural products derived from medicinal plants used in TCM in online literary resources, e.g., PubMed, as well as pertinent monographs [7-10] were searched. A total of 2420 compounds were identified. We compiled 531 of these compounds in a database as a tool for the analysis of molecular modes of action of these natural products [11].

Our interest on natural products from TCM was raised in the 1990's by sesquiterpene lactones of the artemisinin type from *Artemisia annua* L. [12]. We have analyzed the effects of artemisinin-type drugs against cancer cells [for review see 13, 14]. In the course of our studies, we have also analyzed cellular and molecular mechanisms of a panel of other chemically characterized natural products derived from TCM [15-19].

Because of our own focus in cancer research, we mainly concentrate on cancer-related topics of TCM in the present article.

Composite and complex TCM remedies (*fu-fang*) may act in a synergistic fashion to increase therapeutic effects or, on the other hand, may quench side effects on healthy tissues by adverse effects. By means of the current methodology of phytochemistry and pharmacology it is still difficult to identify and prove synergistic or antagonistic effects of dozens of chemical constituents of herbal composite prescriptions.

On the other hand, it is feasible to isolate single or a few active natural products from a medicinal plant and use them as lead compounds to synthesize related derivatives with similar or better pharmacological properties. The principle of this approach has been shown for camptothecin. This natural product was first isolated from *Camptotheca acuminata*, but it is also present in other plant families. Camptothecin can be found in most parts of *C. acuminata*, e.g., roots, root barks, fruits, and leaves, but it is especially abundant in seeds. Much effort has been spent to improve the pharmacological features of camptothecin with respect to increased water solubility and more stable lactone analogues by the design of semi-synthetic derivatives (Fig. 1). The problem with camptothecin is its hydrolyzation to the biologically inactive carboxylate, which binds to serum albumin. Many structural modifications of the different rings of camptothecins have been generated [20, 21]. The most promising derivatives are 9-[(dimethyl-amino)methyl]-10-hydroxy camptothecin (topotecan) and 7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyl oxy camptothecin (irinotecan), which exhibit a much better water solubility than camptothecin [22]. Homocamptothecin shows enhanced plasma stability and a decreased binding to serum albumin. The activity of homocamptothecin can be further enhanced by mono- or bis-fluorination of the quinoline ring of the compound [20].

Camptothecin inhibits topoisomerase I (Fig. 2). Topoisomerase I is required during DNA replication, where it unwinds single DNA strands by inducing single strand breaks to relieve torsional pressure ahead of the replication fork. In the first step, topoisomerase induces strand breaks, and subsequently this enzyme religates the cut strand to restore

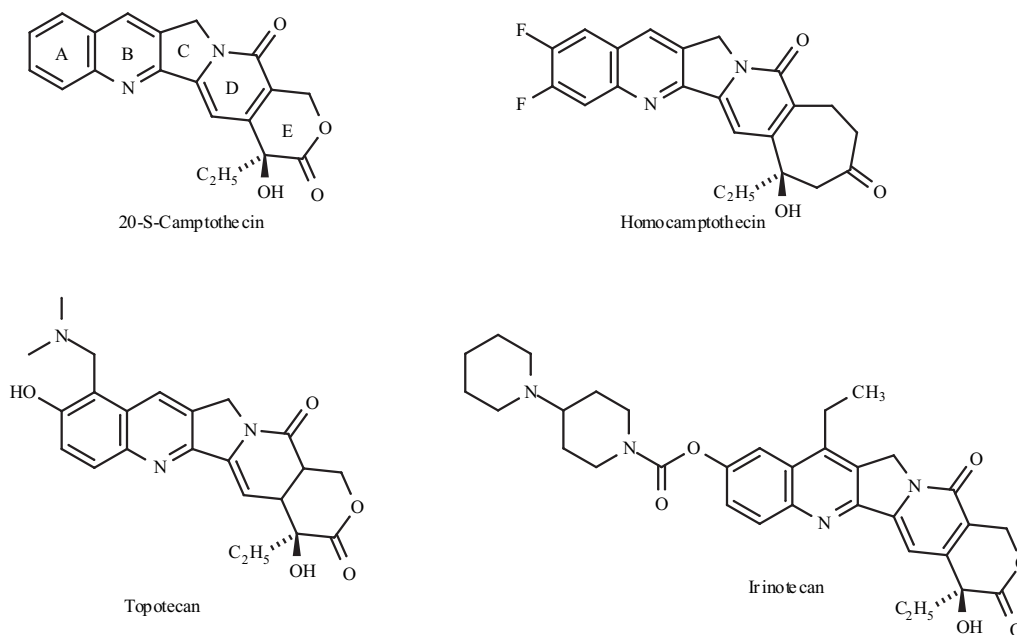


Fig. (1). Chemical structures of camptothecin, topotecan, irinotecan, and homocamptothecin.

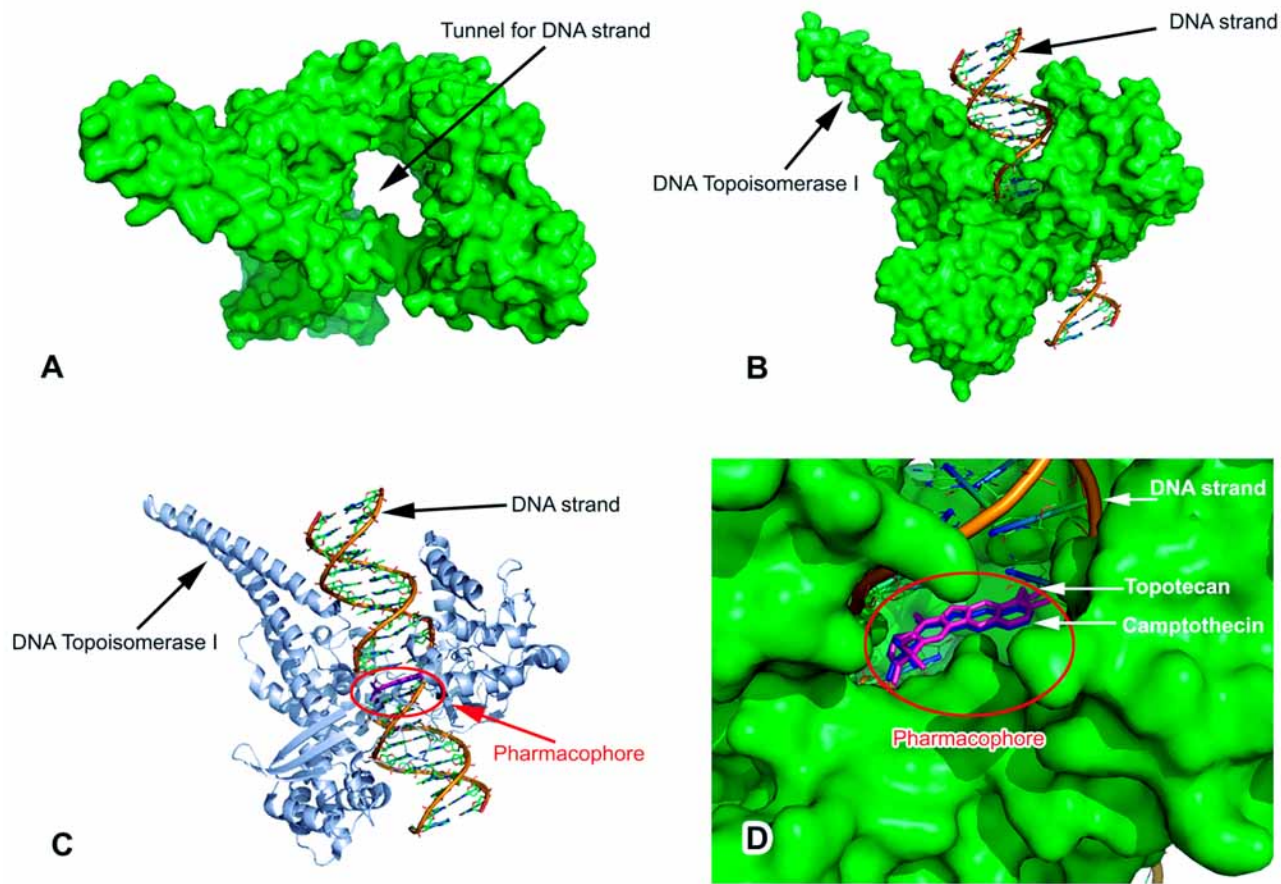


Fig. (2). The crystal structure for human DNA topoisomerase I in covalent complex with 22 base pair DNA duplex and complex with camptothecin (PDB code : 1T8I [74]) and topotecan (PDB code : 1K4T [75]) were retrieved from the protein data bank (PDB; <http://www.rcsb.org>). The figures presented here are prepared using PyMol software (<http://pymol.sourceforge.net>).

A. Surface representation of DNA topoisomerase I crystal structure (in green) exposing the DNA-duplex entry-exit tunnel.

B. Representation of binary complex of Topo I – 22bp DNA duplex with DNA topoisomerase I in green surface representation and 22bp DNA duplex represented in ribbons.

C. Representation of ternary complex with DNA topoisomerase I and 22bp DNA duplex complex with the inhibitors camptothecin and topotecan. DNA topoisomerase I in purple ribbons and DNA in orange ribbons. The pharmacophore is encircled in cyan, and camptothecin and topotecan are represented in blue and purple sticks respectively.

D. Detailed view at the pharmacophore site of DNA topoisomerase I inhibitors: camptothecin in blue sticks and topotecan in purple sticks.

DNA integrity. Camptothecin inhibits the second enzymatic step by stabilizing the cleavable complex between topoisomerase I and DNA. This leads finally to apoptosis [23].

MOLECULAR PHARMACOLOGY AND PHARMACOGENOMICS

A major hurdle of established drugs is their frequent lack of target specificity. Many polyphenols, glycosides, and terpenoids can interact with most proteins and, therefore, exhibit pleiotropic effects [24, 25]. Other compounds are more specific, but do not discriminate between healthy and afflicted organs. This is exemplified by cytostatic drugs, which reveal only poor tumor specificity. Severe side effects in normal tissues prevent treatment with doses sufficient to kill all cancer cells, which in turn fosters the development of drug resistance. Based on the urgent need to develop new drugs with improved features for tumor therapy, researchers' interest in natural compounds was raised. The isolation of natural products and the elucidation of their chemical structure enable pharmacological and molecular biological investigations

comparable to chemically synthesized compounds. The identification of molecular targets of natural products and related signal transduction pathways allows the clarification of modes of action.

The analysis of a novel compound is frequently compromised by the multiplicity of mechanisms, which account for a drug's activity [26-29]. A synopsis of the relevant mechanisms, which influence drug effects allows them to be classified as to whether they act upstream of the actual drug target, at critical target sites, or downstream of them (Fig. 3) [30, 31].

1. Mechanisms acting upstream include transporter proteins for uptake or excretion (i.e., ATP-binding cassette (ABC) transporters, reduced folate carriers, and nucleoside transporters) and drug-metabolizing enzymes that activate, inactivate, or detoxify drugs (i.e., phase I/II enzymes). Metabolizing enzymes and transporter molecules often do not exhibit specificity for certain anti-cancer drugs, but they are operative towards a wide array of different xenobiotic drugs

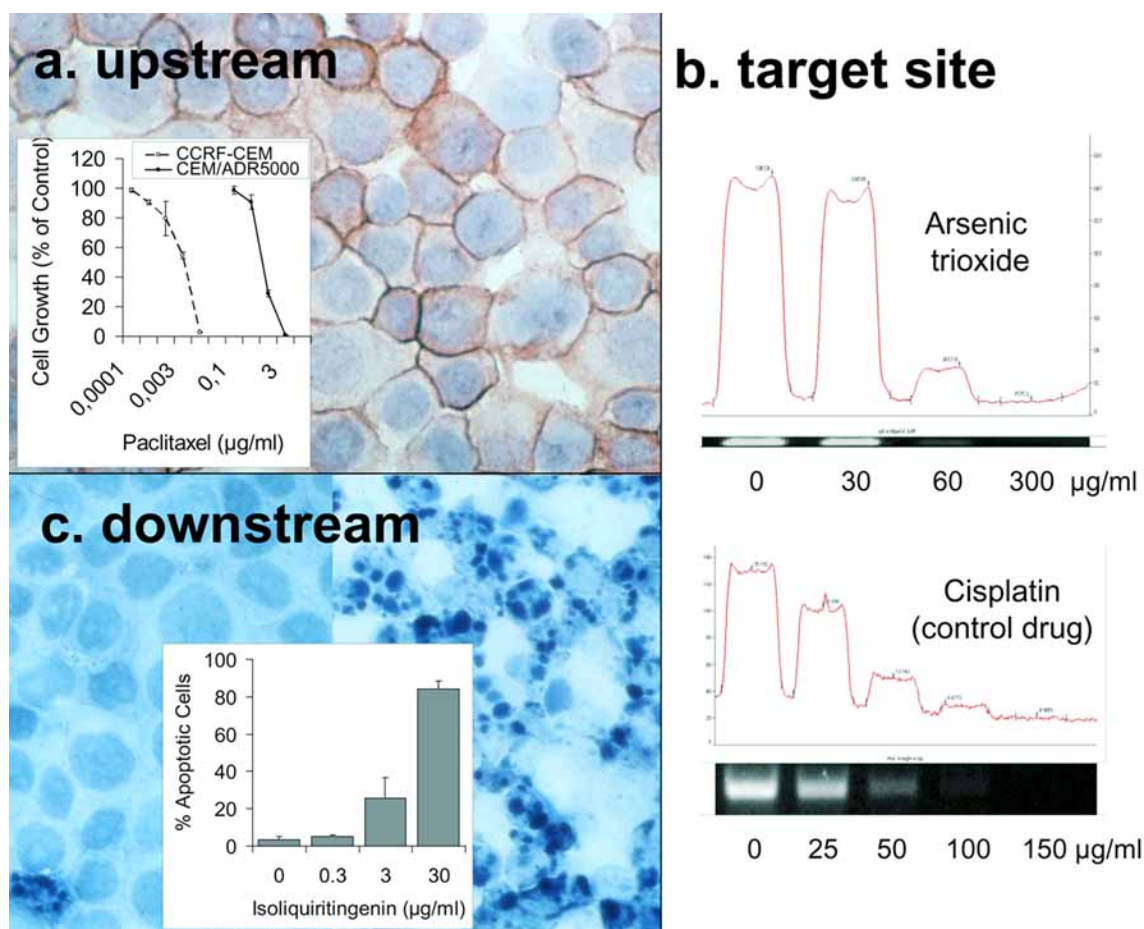


Fig. (3). Multiple mechanisms of resistance of cancer cells towards natural products with activity against cancer cells.

A. Upstream mechanisms: The ABC transporter P-glycoprotein is localized in the cell membrane [25] and extrudes multiple drugs including some derived from TCM out of multidrug-resistant cells. As shown here, P-glycoprotein expressing CEM/ADR5000 leukemia cells were 200-fold more resistant to paclitaxel (from *Taxus chinensis*) than drug-sensitive parental CCRF-CEM leukemia cells (insert in A).

B. Natural products such as arsenic trioxide kill cancer cells by damaging DNA. Among the methods to detect DNA lesions is the PCR stop assay. Since the processivity of Taq polymerase is disturbed by DNA lesions, increasing doses of DNA-damaging agents such as arsenic trioxide lead to decreased PCR amplification. Cisplatin is a well-known DNA-damaging cytostatic agent, which has been used as a control drug. The PCR-stop assays shown here were performed in collaboration with Dr. Dirk Lankenau, University of Heidelberg, Germany.

C. Drugs which bypass upstream and target side resistance mechanisms lethally damage the cancer cell and induce apoptosis as shown for isoliquiritigenin (from *Glycyrrhiza glabra*) in leukemia cells. While untreated control cells reveal an intact nuclear morphology (left picture in panel C), apoptotic cells undergo nuclear fragmentation [76] (right picture in panel C).

including anti-cancer agents. Drug-metabolizing enzymes may influence pharmacokinetics and -dynamics.

- Drug target sites are the DNA (and DNA repair mechanisms) for alkylating agents and platinum drugs, RNA (RNA synthesis inhibitors, i.e., actinomycin D), and specific proteins such as DNA topoisomerases I/II (camptothecins, anthracyclines, and epipodophylotoxins), tubulins (*Vinea* alkaloids and taxanes), or enzymes of DNA biosynthesis (anti-metabolites).
- Mechanisms downstream of the actual drug targets and at distinct intracellular locations are operative after injury by drugs has taken place. The most important downstream mechanism is apoptosis. Its de-regulation may lead to drug resistance and survival of cancer cells.

It is reasonable that the same is true for cytotoxic compounds from TCM (Fig. 3), and pharmacogenomic approaches are helpful in identifying these molecular

determinants [13, 14, 16-19, 32]. DNA and protein microarrays allow the simultaneous analysis of thousands of genes or proteins in a single experiment. Microarray-based profiling has been shown to be helpful to unravel not only possible targets of cytotoxic compounds, but also related upstream and downstream mechanisms, which also contribute to the response of tumor cells to cytotoxic insult. With genome-wide technologies at hand, it is possible to detect novel and sometimes surprising interconnections between pathways involved in drug response. On the other hand, microarray expression data do not only reveal relevant players of drug response, they also show unrelated alterations without causative influence on chemosensitivity. The annotation of genes found by microarray analyses to their functions is a reliable predictor of their role in drug response, since at first sight gene functions for drug response can be more obscure, i.e. negative feedback loops of gene expression may be a cause for counterintuitive regulation [33].

The value of microarray analyses for drug discovery in the field of TCM has impressively been demonstrated for

camptothecin and its derivatives. The microarray-based expression profiling of 149 genes significantly predicted response to camptothecin in a panel of 30 colon carcinoma cell lines [34]. On the level of upstream mechanisms, ABC transporters play an eminent role. KB cervical carcinoma cells selected for resistance to 9-nitrocamptothecin showed a dramatic overexpression of *ABCB1 (MDR1)* and *ABCC2 (MRP2)* in microarray hybridization, while glutathione S-transferase- π was downregulated [35].

Several genes have been identified with the help of microarrays downstream of topoisomerase I as a target interaction site for camptothecin and its derivatives. Zhou *et al.* [36] found that several p53-activated stress-response genes were up-regulated after treatment of HCT116 colon carcinoma cells with camptothecin. The up-regulation of mitosis-related genes was delayed or blocked after camptothecin treatment. The interrupted up-regulation of these genes was directly associated with an arrest of the cells in the G2-phase of the cell cycle. Irinotecan induced specific changes in expression of cell cycle and apoptosis-associated genes in acute myeloid leukemia cells, i.e., surviving-associated genes and death receptor 5 [37]. Cell lines of different tumor types showed a common differential expression of genes functionally related to cell proliferation or apoptosis, e.g., genes of the ATM and ATR checkpoint pathways, JNK pathway, PI3K-Akt-dependent pathway, mitochondrial cell death pathway, endoplasmic reticulum-related apoptosis, and ubiquitin/proteasome-dependent protein degradation pathway [38]. Inoue *et al.* [39] analyzed the sequence-specific synergistic antitumor activity of a combination of 5-fluorouracil and irinotecan in LoVo colon carcinoma cells and found by microarray analyses that changes in the expression of apoptosis-related genes such as Bcl-2 account for the sequence-dependent cytotoxicity of these two drugs.

Another strategy is to predict response or failure of chemotherapy according to specific expression patterns of mRNA species or proteins in clinical tumor samples [28]. As yet, microarray profiling has only been applied in a limited number of clinical investigations, in order to analyze novel markers with prognostic value for response of tumors to treatment with irinotecan and patient survival. Gene expression profiling of colorectal carcinoma revealed that the proto-oncogenes nuclear receptor of T-cells (NOT) and c-fos were up-regulated in irinotecan and doxifluridine-related regimen but not in 5-fluorouracil-related chemotherapy [40]. The genes of five metallothionein isoforms were found to be overexpressed in gastric carcinoma patients not responding to irinotecan treatment [41]. A causative relation was verified by transfection of metallothionein genes into tumor cells, which resulted in resistance to irinotecan compared to control cells. Gene expression analyses in peripheral blood cells of non-small lung cancer patients treated with irinotecan and paclitaxel showed that the genes for protein phosphatase, interleukin-1 α , IgA, and thyrotropin-releasing hormone receptor were independent predictive factors of chemosensitivity [42]. Interleukin-1 β overproduction was found in camptothecin-resistant U87-MG glioblastoma cells [43].

Gene expression profiling is not only important for the identification of novel prognostic factors but also to find predictors of toxicity in normal tissues. Among other side effects of chemotherapy, mucositis occurs frequently in cancer chemotherapy. Bowen *et al.* [44] observed that multiple genes implicated in the mitogen-activated protein kinase (MAPK) signaling pathway (interleukin-1 receptor, caspases, protein kinase C, dual-specificity phosphatase-6) are associated with irinotecan-induced intestinal damage of rats bearing breast cancer. Whether similar results will be obtained in the clinical setting remains to be seen.

PHARMACOGENETICS FOR INDIVIDUALIZED CHEMOTHERAPY

Despite the considerable success of cancer chemotherapy during the past decades, the development of drug resistance of tumors and severe side effects in normal tissues prevent the cure of many cancer patients. Clinicians have developed prognostic markers, which indicate therapy response and treatment outcome. Because the course of the disease can differ considerably with regard to multiple tumors within the same patient and among patients, it is problematic to define an individual prognosis. The reasons for this wide heterogeneity regarding clinical outcome and response to treatment are not understood. Therefore, great efforts have been undertaken 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 with tolerable side effects. In the context of natural products derived from TCM, two scenarios can be envisaged:

- If tumors are unresponsive to established chemotherapy, novel drugs derived from TCM could be used to treat otherwise refractory cancers.
- If TCM drugs would cause adverse effects, other anticancer agents can be used instead.

The proof-of-principle of this concept has been convincingly demonstrated for irinotecan. The pro-drug irinotecan (CPT-11) is converted in the liver to an active metabolite, SN-38 which is a DNA topoisomerase I inhibitor for the treatment of colorectal cancer. UDP-glucuronosyltransferase isoenzyme A1 (UGT1A1) conjugates SN-38 to the inactive SN-38 glucuronide, which is excreted into bile and urine. With reduced capacity for glucuronidation, SN-38 can cause life-threatening diarrhoea provoked by SN-38 mediated enteric injury [45]. Reduced glucuronidation occurs as a consequence of reduced transcription rate due to abnormal dinucleotide-repeat sequences (5-8 repeats) within the TATA box of the *UGT1A1* gene promoter [46]. There was an inverse relationship between the number of TA repeats and the *UGT1A1* transcription rate. This promoter polymorphism was found in patients with Gilbert's syndrome (mostly as a (TA)₇ repeat called UGT1A1*28 allele), a mild form of inherited unconjugated hyperbilirubinemia, and was responsible both for the inherited disease itself as well as for severe toxicity upon CPT-11 treatment. (TA)_n TAA promoter polymorphisms are more frequent in Caucasians than in Asians, in whom missense polymorphisms in the exons were more common (G71R, R367G, Y486D, P229Q) [47, 48]. The UGT1*28 allele was associated with both a reduced area under the curve (AUC) ratio for SN-38 and an increased total bilirubin level pointing to the relevance for pharmacokinetics of SN-38 [49]. The usefulness of the identification of UGT1A1*28 homo- or heterozygotes for the prediction of severe irinotecan toxicity has been shown in clinical studies [50-51]. Font *et al.* [52] reported that 8 of 23 patients with non-small cell lung carcinomas (34%) with the common genotype achieved disease control (partial response or stable disease) compared to 13 of 24 patients (54%) with the variant genotypes. Furthermore, survival in patients with the variant genotypes was higher than in those with the common genotype.

CLINICAL STUDIES

Although a huge number of patients are treated with TCM herbal formulations, randomized clinical trials are still very rare, as recently recognized by Chinese scientists [53, 54]. In

some cases, where the active principles of medicinal plants have been phytochemically identified and pharmacologically characterized, the situation is different because of the presence of a single entity, and so its clinical efficacy has been intensively analyzed in clinical studies all over the world [55, 56]. Again, camptothecins are good examples, which are used for the treatment of colorectal and ovarian carcinoma. A recent meta-analysis based on 602 patients included in phase III trials has shown that combination treatments with irinotecan increase the survival of patients with advanced colorectal cancer compared to treatment regimen without irinotecan [57]. Diflomotecan (BN80915) is an E-ring modified camptothecin

analogue that possesses greater lactone stability in plasma compared with other topoisomerase I inhibitors, which entered clinical phase I and II trials [58, 59].

Paclitaxel, originally isolated from *Taxus brevifolia* (Taxaceae), has also been found in *Taxus chinensis*. Up to date, paclitaxel is the most active anticancer drug used in the clinic. The anti-malarial artemisinin from *Artemisia annua* is also active against cancer cell lines *in vitro* and *in vivo* [60-62]. A less prominent but also promising TCM compound is indirubin from *Indigofera tinctoria* for the treatment of chronic myelocytic leukemia. Ginsenoside Rh2 in ginseng (*Panax ginseng*), daidzein in soybean (*Glycine max*), and acetyl

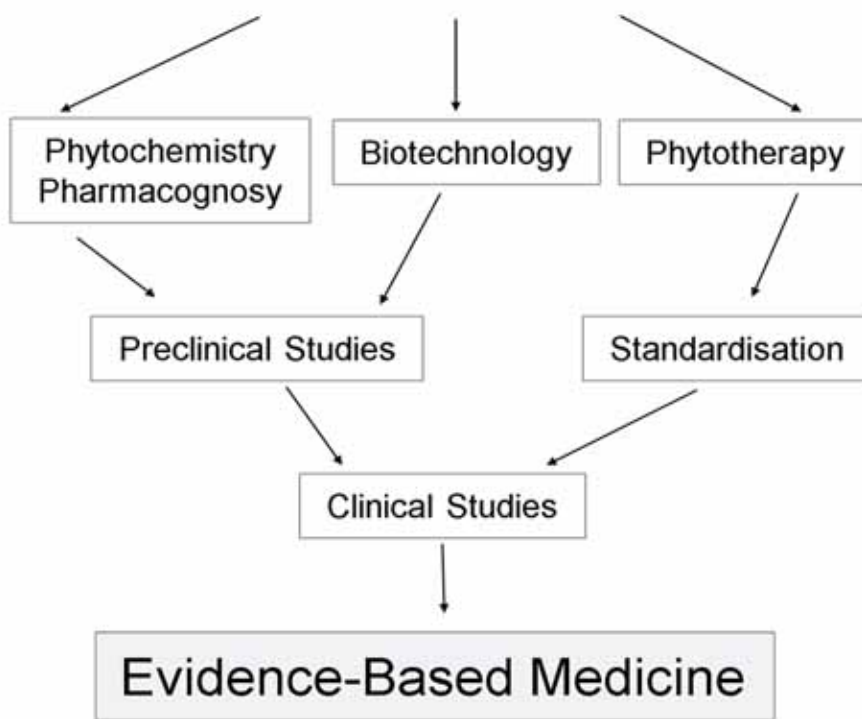


Fig. (4). From plant breeding to evidence-based medicine. Several strategies in the fields of phytotherapy, chemistry, and biotechnology have been developed for the large-scale production of drugs derived from TCM plants and to preserve the natural resources of medicinal plants in the wild. These efforts can be prerequisites for preclinical and clinical studies. Cultivars of *Camptotheca acuminata* are shown.

boswellic acid in *Boswellia serrata resin* are strong inducers of cell differentiation in melanoma cells. Curcumin from *Curcuma longa* represents a chemopreventive agent. Two other TCM compounds, which are under clinical investigation for cancer treatment, are homoharringtonine from *Cephalotaxus harringtonia* and arsenic (III) oxide from the mineral ore, arsenopyrite [63]. Clinical studies with homoharringtonine have demonstrated promising results initially in China and later on in the United States [64-66].

BIOTECHNOLOGY

Chinese medicinal herbs growing in the wild cannot fulfill the needs for sustainable health care, either in China or worldwide. Therefore, technological solutions to avoid the

extinction of endangered plant species are of utmost importance (Fig. 4).

While in some cases, it may be worthwhile to synthesize natural products chemically in the laboratory, many chemical structures are too complex and not easily amenable to chemical synthesis. Therefore, several biotechnological approaches have been developed during recent years [67]:

1. Agricultural Approaches

- to cultivate wild-type plants in fields and greenhouses;
- to breed classical high-yield cultivars using traditional plant breeding.
- to cultivate and optimize transgenic plants.

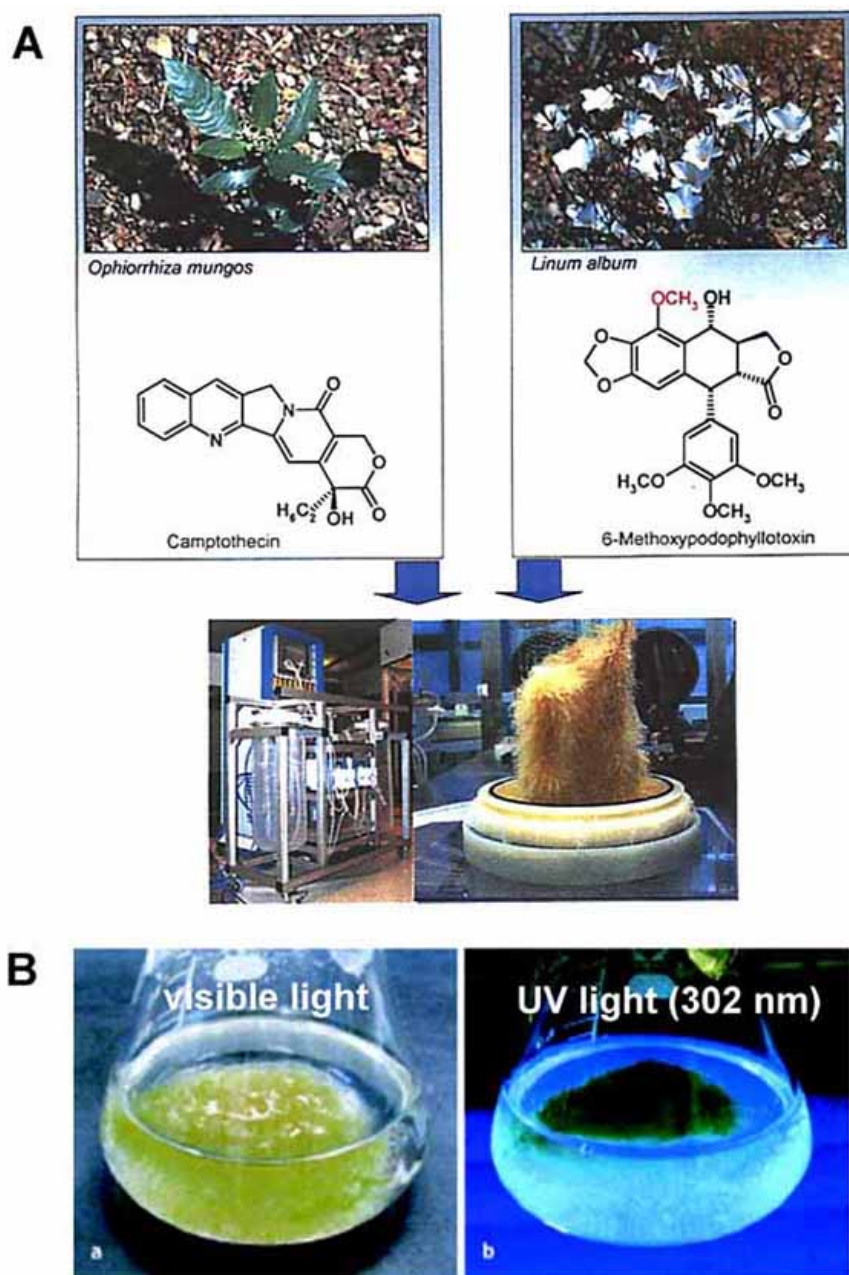


Fig. (5). Hairy root cultivation of *Ophiorrhiza mungos* for the production of camptothecin.

A. Production of hairy roots in a MIST fermenter (Rootec Company) used for the production of camptothecin and podophyllotoxin [70].

B. Camptothecin is partly released into the culture medium. Camptothecin is fluorescent at 302 nm, therefore the medium appears blue in color.

2. Biotechnological Approaches for the Large-Scale Production of Natural Products

- Root cultures or hairy root cultures of medicinal plants; they are generated by infection of roots with *Agrobacterium rhizogenes*;
- Cell cultures (suspension and callus cultures) derived from medicinal plants;
- The expression of biosynthetic pathways for natural products by recombinant microorganisms, i.e., *Escherichia coli*, *Saccharomyces cerevisiae* etc. When the genes of the corresponding plant enzymes are transferred to bacteria or yeast, it is possible to reconstruct the biosynthesis pathways of natural products in these organisms. However, since most pathways comprise several enzymes (the corresponding genes are often unknown), this approach has not been successful so far [67].

An intriguing example, how sustainable production of natural products can be achieved represents camptothecin. While the generation of callus cultures and cell cultures of non-differentiated callus tissue can easily be achieved, the yield of secondary metabolites is frequently low. The yield of camptothecin in callus cultures or cell cultures of *Camptotheca acuminata* is disappointing [68, 69]. Probably, the enzymes necessary for the biosynthesis of secondary metabolites are downregulated in undifferentiated tissues such as callus [70]. An alternative represents the culture of differentiated tissues such as roots or shoots. Bioreactors have been developed for root or shoot cultures on a commercial scale. The infection of root cultures with *Agrobacterium rhizogenes* leads to the formation of hairy roots with improved production of secondary metabolites. Camptothecin is not only found in *Camptotheca acuminata*. Other plant species also produce this compound, i.e., *Nothapodytes foetida*, *Ophiorrhiza pumila*, and *Ophiorrhiza mungos*. Camptothecin production in *Ophiorrhiza mungo* has been successfully demonstrated in one of the author's laboratories (M.W.) (Fig. 5). Taking advantage of a bioreactor system for the large scale production of hairy roots [71], normal root and hairy root cultures produced considerable amounts of camptothecin (approx. 1-3 mg/g dry weight) [72]. These yields correspond to camptothecin levels in *C. acuminata* seeds. Hairy root cultures are maintained on racks continuously sprayed with culture medium. Since camptothecin is released into the culture medium, products can be harvested in a semi-continuous and continuous fashion. Camptothecin is partly released into the culture medium, a process which can be enhanced by biogenic elicitors. HPLC and LC-MS analyses revealed camptothecin as the main product [23, 24]. The genes of the key enzymes responsible for the biosynthesis of camptothecin have been cloned from hairy roots of *Ophiorrhiza pumila*, e.g. strictosidine synthase, and NADPH:cytochrome P450 reductase. Heterologous expression of the cDNAs coding for these enzymes have been expressed in *E. coli* cells and yielded functional recombinant proteins [73]. This is an important step towards genetic engineering for recombinant camptothecin biosynthesis for large-scale production. These examples demonstrate that biotechnological approaches represent attractive alternatives to field cultivation.

CONCLUSIONS AND PERSPECTIVES

Isolation of natural products and elucidation of their chemical structure enable pharmacological and molecular biological investigations comparable to chemically synthesized compounds. As exemplified for camptothecin, natural products can serve as lead compounds for the

bioactivity-based generation of semi-synthetic derivatives. The identification of target molecules (i.e. topoisomerase I in the case of camptothecin) represents the basis for the development of rational treatment strategies for natural products from TCM.

This also opens avenues for the prediction of individual response of a cancer patient to therapy. While the statistical probability of therapeutic success is well-known for larger groups of patients from clinical therapy trials, it is, however, still not possible to predict which individual cancer patient will respond to chemotherapy. It would be, therefore, of great value for patients to know, whether or not a tumor would respond to the proposed therapy or whether undesirable side effects may occur [27, 30]. Single nucleotide polymorphisms in the *UGT1A1* gene are of utmost importance for otherwise unpredictable toxicity towards irinotecan. This concept of individualized tumor therapy is also of great importance for other small molecule inhibitors derived from TCM [12, 13, 15-18].

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