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Review

Pharmacogenomics of a Traditional Japanese Herbal Medicine (Kampo) for Cancer Therapy

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Abstract. *In the present review, we give a short introduction into the history, philosophy and traditional diagnosis and therapy of Kampo, which has its origins in traditional Chinese medicine. The main focus is on pharmacogenomics of natural products derived from Kampo medicinal plants, with special emphasis on cancer treatment. One of these natural products with profound cytotoxicity against tumor cell lines is shikonin from the medicinal plant Lithospermum erythrorhizon. This compound has been selected to demonstrate how molecular determinants of response of tumor cells to Kampo-derived natural products can be investigated by microarray-based approaches. Synthetic or semi-synthetic derivatives of natural products from Kampo medicine may lead to novel drugs with improved features for cancer treatment. Kampo-derived natural products represent a valuable reservoir for individual tumour treatment strategies in the future.*

History of Kampo

The present review gives a short introduction into the history, philosophy and traditional diagnosis and therapy of Kampo. The main focus is on pharmacogenomics of natural products derived from Kampo medicinal plants. Therefore, the reader is also referred to recent comprehensive reviews (1-5).

Kampo has its origins in traditional Chinese medicine (TCM). In the 5th and 6th century, Chinese and Korean monks, as well as envoys of the Japanese emperor's court, came to Japan and brought TCM along. These medicinal 'recipes' spread all over Japan in the following centuries. There was consequently a need for their compilation in a uniform and systematic system. As a result, the medical textbook "Ishinho" (Essence of Healing) was written by Yasunori Tanba in the year 984, mainly based on the book "Shang Han Lun" (Survey of Febrous Diseases) by Chang Zhong-jung (142-220 AD). The "Ishinho" textbook was the starting point for the development of traditional Japanese medicine independently from TCM. As the flora in Japan differs from that of China, an independent development of Japanese herbal medicine was further fostered. The general TCM system was transferred to Japan, but indigenous plants were used by the physicians. In the 16th century, sailors from Portugal and the Netherlands traveled to Japan. They established trade stations and hospitals in Japan and dispersed Western medicine over the country. At that time, traditional Japanese medicine was called "Kampo" to distinguish it from the Dutch medicine, "Rampo" ("ran"="oranda"=Holland). From the 17th century, the Toyama province gained a considerable reputation for its Kampo medicinal products, which were traded all over Japan. At the beginning of the 18th century, the Koho School was established, which was a milestone in the development of Kampo medicine. One of its most famous representatives was Yoshimasu Todo (1702-1732 AD) who revised the recipes of Shang Han Lun. He also focused on abdominal palpation, which had lost its relevance in TCM diagnostics. Another Koho teacher was Toyo Yamawaki, who introduced the dissection of subjects in 1754. In his anatomical textbook "Zoshi" (On the Internal Organs), he disproved parts of the TCM theory. Another important milestone was a breast cancer operation performed by Sheishu Hanaoka in 1805. He used herbal mixtures containing *Datura metel* (thorn apple) and *Aconitum japonicum* (monk's hood) to achieve general anesthesia of the patients to be operated on. From the second half of the

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19th century, Japanese medicine was reformed according to the Western archetype, especially that of German medicine. As a consequence, Kampo medicine lost its influence and was almost forgotten. Not until the second half of the 20th century, Kampo medicine experienced a thriving revival in Japan and also in the Western world due to an increasing interest in alternative and complementary medicine.

Philosophy of Kampo

The basic concept of Kampo medicine is comparable to TCM: A healthy body is in a harmonic balance and diseases originate from the disturbance of this harmony. Disharmony is caused by external factors (cold, heat, moisture, dryness, fire and wind) or internal factors (joy, grief, worry, fear, anxiety and rage). The harmonic balance in health is influenced by three parameters: i) the vital energy *Qi*, which penetrates the entire body. It flows in all organs. Excess as well as deficiency of *Qi* causes diseases; ii) the complementary and antithetic principles *Yin* and *Yang*. An excess of one principle causes a deficiency of the other one; iii) the relation of the five elements (wood, fire, earth, metal and water) to each other. All living and non-living things on this world are composed of these five elements. In human beings, the five elements are allocated to five organs (liver, heart, spleen, lung, kidney), which are in balanced interaction with one another.

Diagnosis and Therapy of Kampo

Generally, a Kampo physician takes advantage of both Kampo and Western medicine diagnostic tools. The Kampo criteria comprise a talk with the patient, an audio-olfactorial investigation, investigation of the tongue and skin, and the palpation of the forearm and abdomen. The physician diagnoses the disease pattern (*Sho*) and assigns it to a corresponding therapy (*Ho*). Medicinal herbs play a major role in Kampo medicine, although other aspects such as life style *etc.* are also taken into consideration.

Medicinal Herbs in Kampo

Over 200 Kampo recipes have been reported, which are mixtures of two to 15 components. About 350 single components are used for these recipes. Most of them are medicinal herbs, but fungi, animal components and minerals are also used. About 120 of these crude drugs are listed in the Japanese Pharmacopeia and one third of them are also listed in WHO monographs (6). The quality criteria of these crude drugs are defined and health insurance pays for their prescription.

Because Kampo medicines are in most cases mixtures, and their action may rely on the interaction of the single constituents of these mixtures, Kampo medicine is used in a wide array of applications. However, it should be taken into account that Kampo medicine may have antagonistic or synergistic interactions with Western drugs or certain foods by affecting the activity of phase I/II enzymes and other enzymes. Toxic side-effects have been reported such as acute, sub-acute or chronic toxicities, teratologic or reproductive toxicities, and mutagenic effects.

Kampo at the Interface of Western Academic Life Sciences

It is a widely recognized fact that many drugs and pharmacologically active compounds are derived from natural resources such as medicinal plants. Some prominent examples 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*.

Hence, it is quite reasonable to search for novel drug molecules in medicinal plants used in Kampo (Figure 1). This is the task of different disciplines such as phytochemistry, pharmacognosy, and pharmacology, which may deliver valuable results together with modern technical platforms of molecular biology, pharmacogenomics and proteomics, as well as systems biology. The present review focuses on preclinical studies and especially on pharmacogenomics and cancer therapy.

Apart from preclinical research, clinical and biotechnological work has to be done to bring plant-derived compounds to the market. Clinical studies are necessary to provide evidence for the efficacy of Kampo-derived small molecules as well as medicinal plant preparations (phytotherapy) according to internationally acknowledged quality criteria. Currently, one of the most thriving novel concepts in cancer research is the individualization of therapy. While the statistical probability of therapeutic success is well-known for larger groups of patients from clinical therapy trials, it is, however, not possible to predict which individual cases of cancer patients 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. If the tumor is resistant, while there is no influence on the tumor growth, the therapy will only cause toxic effects in normal tissues; hence, another, more effective regimen could be applied or in the worst case, chemotherapy could even be skipped. This concept of individualized tumor therapy (7-11) is also of great importance for natural products (12-17). Kampo medicine and Kampo-derived compounds may provide exquisite possibilities for such tailor-made treatment strategies.

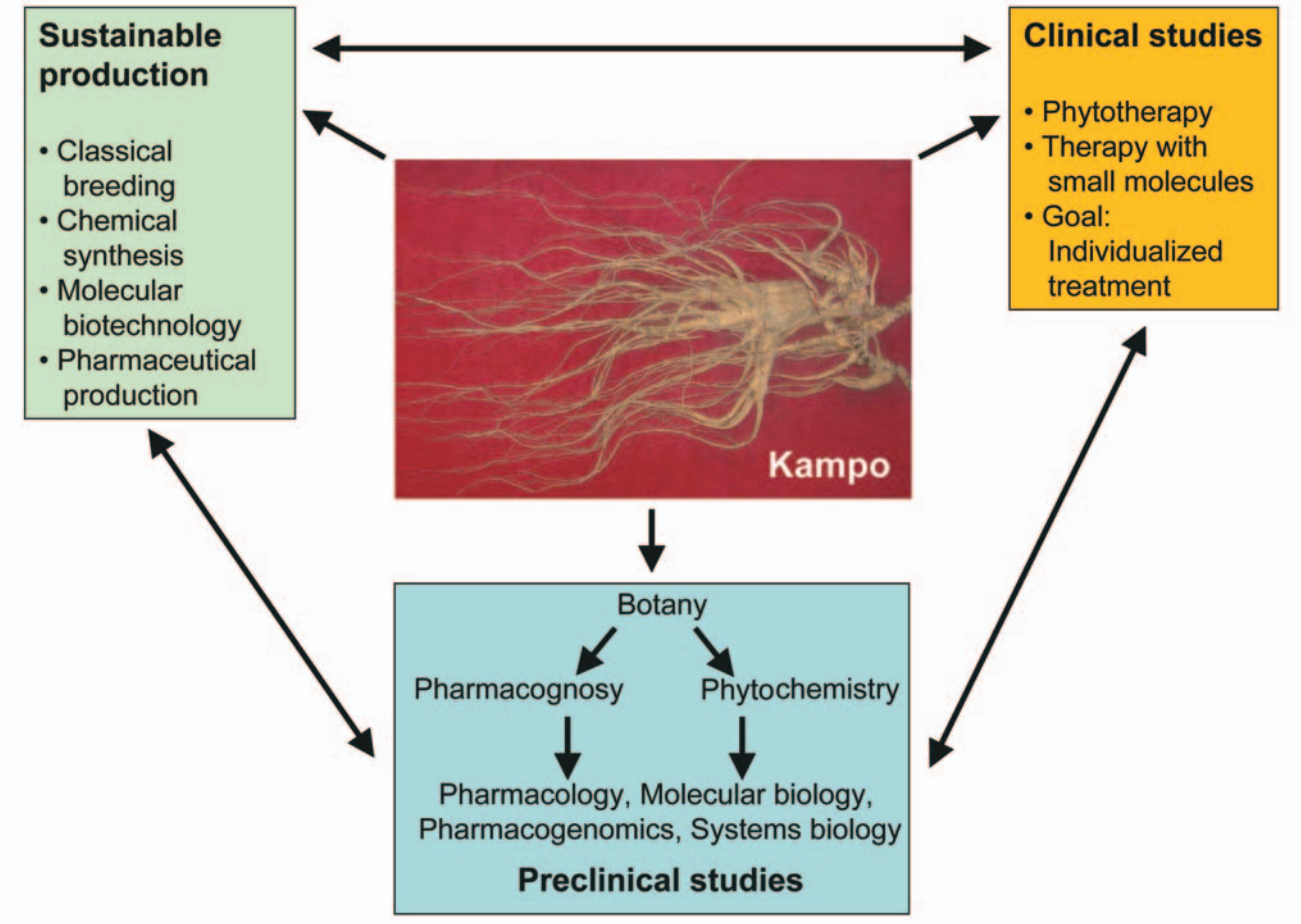

Figure 1. *Kampo medicine at the interface of Western academic life sciences.*

General problems of pharmacologically active compounds from medicinal plants concern sustainability and the preservation of natural resources. Plants harvested from the wild cannot fulfill the demands of cancer treatments in the long run for obvious reasons. Therefore, strategies for their large-scale production have to be developed. Agricultural approaches (classical breeding in fields and in greenhouses), chemical synthesis of natural products in the laboratory, and techniques of molecular biotechnology (hairy root cultures, cell cultures, engineered micro-organisms for the biosynthesis of natural products) have been described during the past few years.

Kampo Medicines in Cancer Treatment

In cancer treatment, Kampo medicine has been used to promote physical reconditioning and to reduce adverse effects after chemotherapy or radiation therapy, enhancing the quality of life. The use of Kampo medicines as chemotherapeutic agents is quite reasonable when considering the concept of Kampo medicine, which expresses its effects in pathological conditions of imbalanced homeostasis, but not in physiological ones of balanced homeostasis. A tumor is a final status of imbalanced homeostasis. Therefore, Kampo medicine aims to increase tumor specificity while minimizing adverse effects on normal tissues.

The regimen in Kampo medicine is selected and its dose is adjusted on the basis of the individual difference in responsiveness to it. In the concept of Kampo medicine, the absorption, distribution, metabolism and excretion of medicines differ between patients. This concept correspond to a principle of personalized medicine based on pharmacokinetics and pharmacodynamics in Western medicine. In order to maximize its effects and to minimize adverse effects, Kampo medicines as chemotherapeutic agents should be appropriately selected for an individual patient. A promising approach is the prediction of responsiveness prior to treatment on the results of the gene expression profile of tumor tissue, as described in this review.

Cytotoxic Activity of Kampo-Derived Natural Products

Classical approaches to identify the molecular modes of action of natural products are time-consuming and costly. The advent of "-omics" technologies (*e.g.* genomics, proteomics, metabolomics) allows identification of possible targets and determinants of cellular response to treatment with investigational drugs.

Most medicinal plants used in Kampo medicine have been described in the literature (18, 19). As a starting point, we have screened the literature for compounds isolated from medicinal plants by phytochemical means. We focused only on small molecular weight, macromolecules (*e.g.* peptides) were not considered. Next, we determined, whether these compounds are included in the database of the Developmental Therapeutics Program of the National Cancer Institute (NCI), Bethesda, USA (http://dtp.nci.nih.gov). We identified 40 chemically characterized natural products (Table I). These compounds have been tested for their cytotoxic activity towards a panel of cell lines of different tumor types (leukemia, melanoma, brain tumor, carcinoma of colon, breast, ovary, kidney, lung, or prostate) (20). All compounds were tested by means the sulforhodamine B assay. Sulforhodamine B binds to proteins and is widely used for chemosensitivity testing (21). The IC_{50} values of these drugs are given in the NCI database. Table I shows the mean IC_{50} values for these 40 natural products of the entire cell line panel. Six compounds showed considerable cytotoxicity with mean $\log_{10}IC_{50}$ values of <-5.0 M, while 12 natural products showed intermediate mean $\log_{10}IC_{50}$ values (–4.7094 to –4.2226 M). A further 22 of these natural products revealed no or only weak cytotoxicity (mean $\log_{10}IC_{50}$ values of ≥–4.2 M).

The ten most cytotoxic natural products derived from Kampo medicinal plants were further analyzed. We were interested to determine which tumor types were sensitive and which ones were resistant to these compounds. The mean IC_{50} values for cell lines of each tumor type are depicted in Figure 2. Interestingly, leukemia cell lines were most sensitive, whereas brain tumor cell lines were most resistant to natural products derived from Kampo medicine. These results reflect the situation in the clinic with established cytostatic drugs. While a considerable proportion of leukemia patients can successfully be treated with standard chemotherapy (in the case of juvenile leukemia up to 80%), brain tumors have a poor prognosis and are largely drug resistant. Other tumor types such as lung cancer or melanoma, which are clinically resistant to standard chemotherapy, showed intermediate mean $\log_{10}IC_{50}$ values to natural products from Kampo medicine. This indicates that these compounds might have the potential for being valuable adjuncts to current chemotherapy protocols. Interestingly, at least in this panel of natural products, cell lines of a tumor type which were sensitive to one compound were not found to be resistant to another natural product and vice versa. The most cytotoxic compound was shikonin (Figure 3). Shikonin is a major component of zicao (purple gromwell, the dried root of *Lithospermum erythrorhizon*), a Chinese herbal medicine with various biological activities. All further analyses were, therefore, performed with this natural product.

Molecular Determinants of Cellular Response to Shikonin

We performed a COMPARE analysis with standard anti-tumor agents included in the NCI database to identify drugs whose IC_{50} values correlate with those of shikonin. COMPARE analyses allow the production of rank-ordered lists of cytotoxic compounds tested in the NCI cell lines. The methodology has been described in detail (22). Briefly, every set of IC_{50} values of a compound in the panel of NCI cell lines was ranked for similarity to the IC_{50} values for shikonin. To derive COMPARE rankings, a scale index of correlation coefficients (R-values) is created. Compounds with COMPARE correlation coefficients of $R>0.7$ are listed in Table II. Many of these drugs are either DNA-damaging alkylating agents (pyrimidine acetaldehyde) or antimetabolites (fluorodopan, rifamycin, asalex). Although the major target of these compounds is the inhibition of nucleic acid biosynthesis, many antimetabolites are also known to induce DNA damage. It is therefore, reasonable to speculate that shikonin might also damage DNA.

As a further step, we performed COMPARE analyses of the IC_{50} values for shikonin and the mRNA expression of 9706 genes of the NCI cell lines to produce scale indices of correlation coefficients. The mRNA expression values were selected from the NCI database. The mRNA expression was determined by microarray analyses (23, 24). We performed a standard COMPARE analysis in which cell lines most inhibited by shikonin (lowest IC_{50} values) were correlated with the lowest mRNA expression levels of genes. The genes identified by this approach are shown in Table III. These genes may be considered as possible candidate genes which determine cellular resistance to shikonin. Furthermore, reverse COMPARE analyses were carried out, which correlated the most inhibited cell lines with the highest gene expression levels (Table III). This approach provided genes that determine cellular sensitivity to shikonin.

The genes identified with this analysis have not been assigned to drug sensitivity or resistance as of yet indicating that the molecular determinants of response to shikonin are different from those of established anticancer drugs. Interestingly, a number of gene products are involved in transcriptional processes (*ZNFN1A1, HCLS1, MYB, NACA,*

Table I. *Cytotoxicity towards the NCI tumor cell line panel of natural products derived from medicinal plants used in Kampo medicine.*

Compound	$\log_{10}IC_{50}$ (M)	MW	Medicinal plant
Shikonin	–6.0 (±0.0628)	288	*Lithospermum erythrorhizon*
Coptisine chloride	–5.8549 (±0.0554)	356	*Coptis japonica*
Protodioscin	–5.5307 (±0.0462)	1049	*Tribulus terrestris*
Betulinic acid	–5.130 (±0.0304)	457	*Zizyphus jujuba*
Curcumin	–5.0854 (±0.0345)	368	*Curcuma longa*
(+)-α-Viniferin	–5.0411 (±0.0421)	679	*Paeonia lactiflora*
Ginsenoside Rh2	–4.7094 (±0.0092)	623	*Panax ginseng*
Berberine chloride	–4.7089 (±0.0644)	384	*Coptis japonica*
Chrysin	–4.7020 (±0.0169)	254	*Eucommia ulmoides*
Emodine	–4.6118 (±0.0249)	270	*Polygonum multiflorum*
Lutein	–4.5951 (±0.0311)	569	*Triticum aestivum*
Apigenin	–4.5750 (±0.0181)	270	*Platycodon grandiflorum*
Medicarpin	–4.5661 (±0.0229)	270	*Glycyrrhiza galbra*
Glycyrrhetic acid	–4.450 (±0.0227)	823	*Glycyrrhiza galbra*
Nobiletin	–4.3971 (±0.0315)	402	*Citrus subcompressa*
Ergosterol	–4.3564 (±0.0263)	397	*Polyporus umbellatus*
Resveratrol	–4.3027 (±0.0452)	228	*Paeonia lactiflora*
Quercetin	–4.2226 (±0.0283)	302	*Eucommia ulmoides*
Ginsenoside RD	–4.1833 (±0.0230)	1894	*Panax ginseng*
Arctigenin	–4.1724 (±0.0448)	372	*Arctium lappa*
Astragaloside II	–4.1007 (±0.0208)	829	*Astragalus membranaceus*
Isopimpinellin	–4.0714 (±0.0347)	246	*Angelica acutiloba*
Palmatine chloride	–4.0490 (±0.0186)	388	*Coptis japonica*
Scopoletin	–4.0403 (±0.1548)	192	*Lycium chinense*
Aconitine	–4.0269 (±0.0199)	648	*Aconitum carmichaeli*
3,4-Hydroxycinnamonic acid	–4.0197 (±0.0120)	164	*Triticum aestivum*
Formononetin	–4.0187 (±0.0179)	268	*Glycyrrhiza galbra*
Xanthotoxin	–4.0159 (±0.0106)	216	*Angelica acutiloba*
Crocetin	–4.0104 (±0.0104)	328	*Gardenia jasminoides*
Ginenoside RG1	–4.0104 (±0.0104)	1001	*Panax ginseng*
Daucosterol	–4.010 (±0.0099)	577	*Astragalus mongolicus*
Sparteine	–4.0 (±0)	332	*Rehmannia glutinosa*
Ferulic acid	–4.0 (±0)	194	*Cnidium officinale*
Naringin	–4.0 (±0)	581	*Citrus paradisi, Primus persica*
Vanillin	–4.0 (±0)	152	*Gastrodia elata*
Ginsenoside A1	–4.0 (±0)	801	*Panax ginseng*
Paeoniflorin	–4.0 (±0)	480	*Paeonia lactiflora*
β-Sitosterol	–4.0 (±0)	415	*Scrutellaria baicalensis*
D-Amygdalin	–3.6610 (±0.1219)	457	*Primus persica*
DL-Amygdalin	–3.648 (±0)	457	*Primus persica*

GFI1, TEAD1, RBM9) or are structural constituents of ribosomes (*RPL5, RPL9, RPL15A, RPL34, RPS12*). This is another hint that nucleic acids may play a major role for the mode of action of shikonin in addition to the cross resistance profiling shown in Table II. Other genes contribute to the regulation of signal transduction (*GIT2, WAS, DOCK2, GNA11, GNG12, ARHE, SH3BP4, TBC1D8*), which might also be a relevant mechanism for the determination of cellular response to cytotoxic drugs. Further studies are warranted to clarify their causative relevance for cellular response to shikonin.

The genes obtained by standard and reverse COMPARE analyses for shikonin were subjected to hierarchical cluster analysis to obtain a dendrogram, where the cell lines are arranged according to their expression profile of these genes. The dendrogram for shikonin can be divided into five major cluster branches (Figure 4). The $\log_{10}IC_{50}$ values for shikonin were then used as a cut-off threshold to define cell lines as being sensitive or resistant. As can be seen in Table IV, the distribution of sensitive and resistant cell lines was significantly different between the branches of the dendrogram, indicating that cellular response to shikonin was predictable using the mRNA expression of these genes.

These analyses gave reason to believe that shikonin may affect DNA and that DNA is a target molecule for

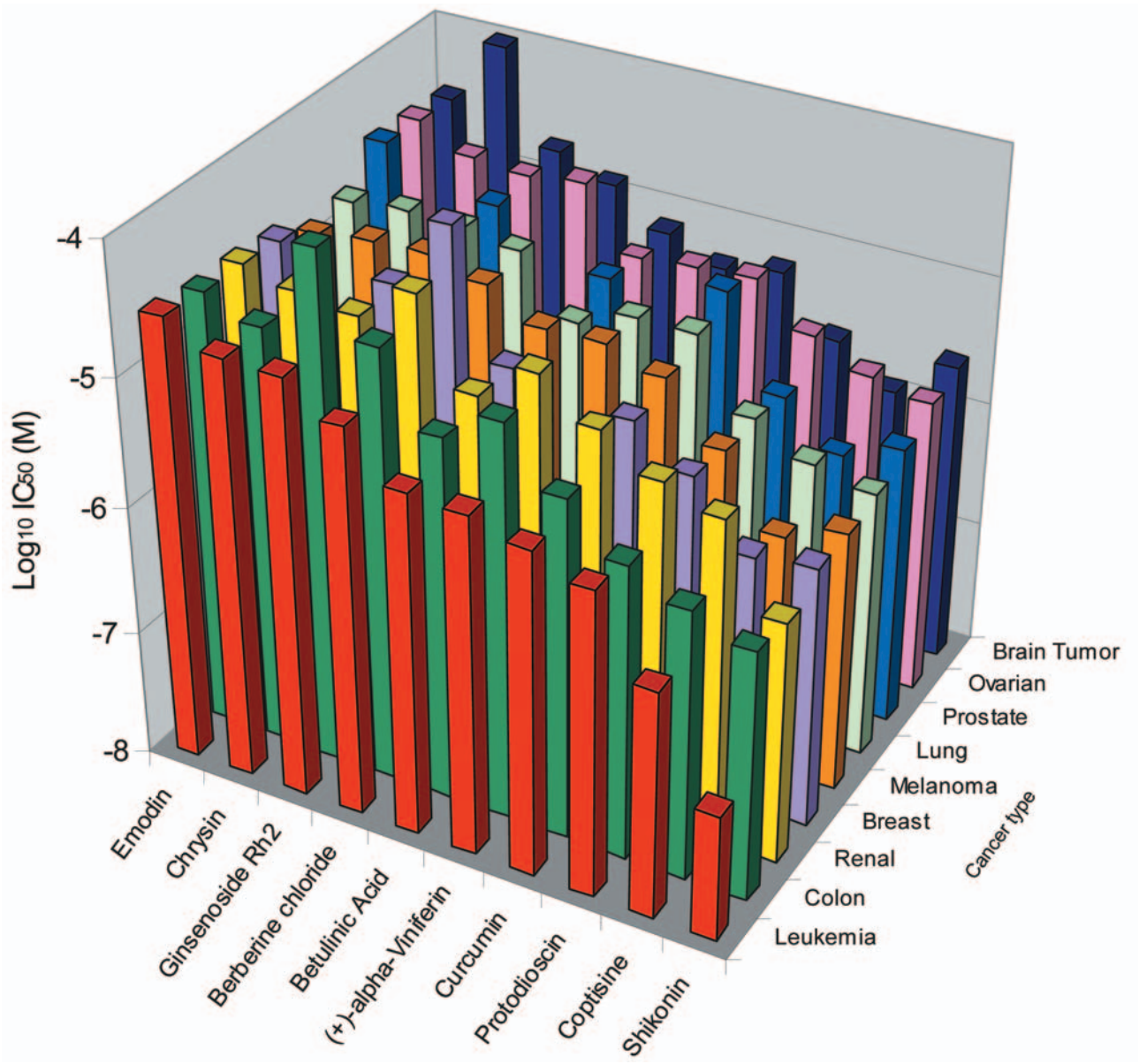

Figure 2. *Mean $log_{10}IC_{50}$ values of 10 natural products derived from medicinal plants used in Kampo medicine for tumour cell lines of the NCI drug screening panel as assayed by the sulforhodamine B test.*

shikonin's cytotoxic activity against tumor cells. This is in accord with other authors, who found that shikonin generates reactive oxygen species and electrophilic molecules (25). Bioreductive alkylation reactions have also been proposed (26). All these reaction modes have the capability of damaging DNA. Furthermore, the inhibition of DNA topoisomerase I and II and telomerase by shikonin has been reported (27-29). Both reaction types induce DNA damage. Topoisomerases are involved in DNA replication, transcription and recombination. The identification of ribosomal proteins and transcription factors by microarray and COMPARE analyses (Figure 3 and Table III) points to an interference of shikonin with the nuclear replication, transcription and recombination machinery.

In light of the numerous molecular determinants contributing to the cytotoxicity of shikonin, it comes as no surprise that the bioactivity of shikonin is not restricted to anti-tumor effects. This compound also possesses anti-microbial, anti-inflammatory and anti-thrombotic features, as well as beneficial effects for wound healing (30).

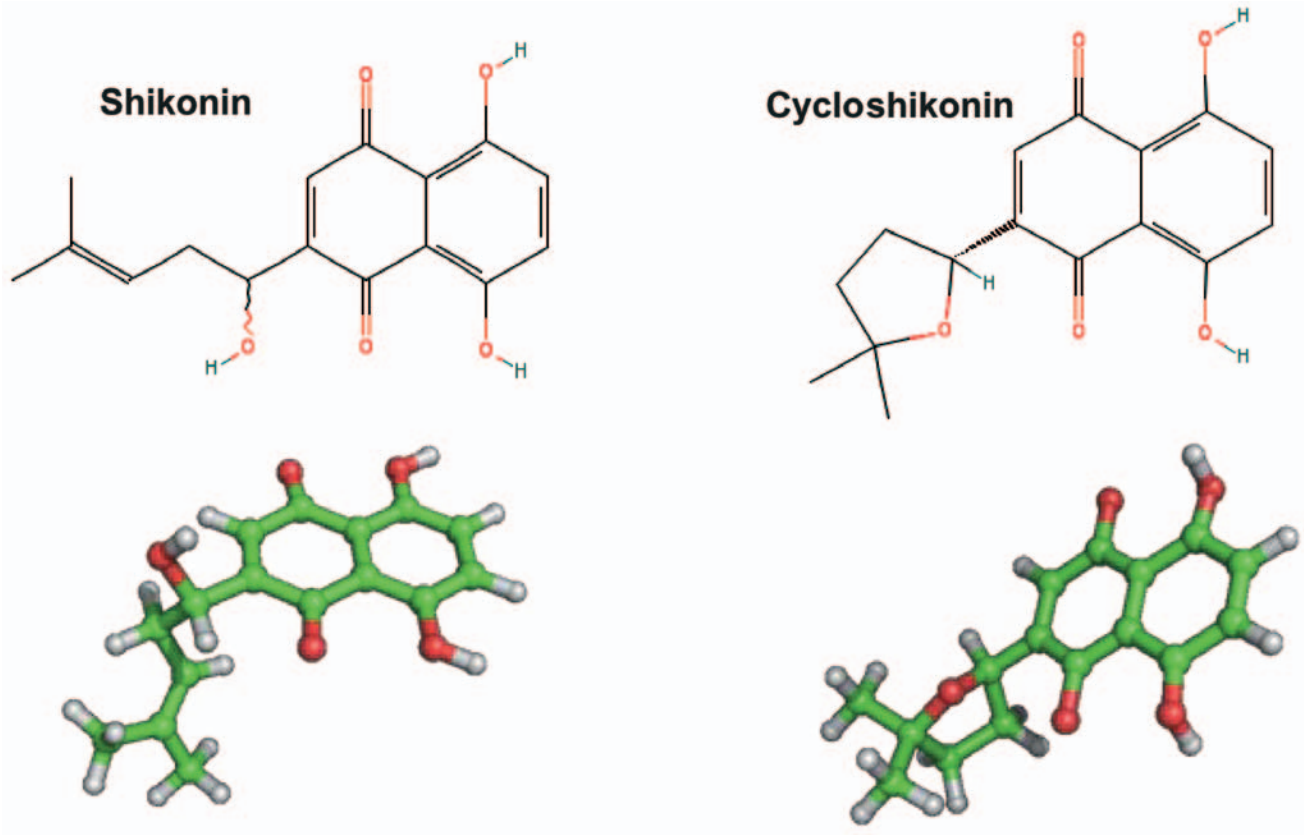

Figure 3. *Chemical structures and three dimensional representation of shikonin and cycloshikonin.*

Derivatization of Shikonin

Shikonin has served as a lead compound for the generation of several synthetic or semi-synthetic derivatives in an effort to improve pharmacological properties, *i.e.*, water solubility, stability, cytotoxicity, Examples are cycloshikonin (Figure 3), acetylshikonin, desoxyshikonin, teracylshikonin and others. Cycloshikonin and desoxyshikonin are also metabolites, which appear during biotransformation of shikonin in the gastrointestinal tract (30). As shown in Figure 5, the cytotoxicity of cycloshikonin was tightly correlated to shikonin in the NCI tumor cell lines ($p=5.94x10^{-13}$, R=0.7686; Spearman's rank correlation test). If the IC_{50} values for cycloshikonin were correlated with the microarray-based gene clusters obtained for shikonin (Figure 4), a significant relationship was observed. As shown in Table IV, the response of cell lines to shikonin and cycloshikonin was similar in the five different clusters. This indicates that the same genes predicting sensitivity or resistance to shikonin also determine cellular response to cycloshikonin.

Table II. *Correlation of log_{10} IC_{50} values for shikonin to $log_{10}IC_{50}$ values for compounds of the NCI Standard Agents Database identified using COMPARE analysis in 60 NCI cell lines.*

Compound	Compare coefficient	Drug class
Fluorodopan, Ftorpan	0.790	Antimetabolite
Pyrimidine acetaldehyde derivative	0.751	Alkylating agent
Rifamycin	0.716	Antimetabolite
Asalex, Asaley (L-Leucine derivative)	0.702	Antimetabolite

Perspectives

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 target molecules relevant for diseases allows screening for

Table III. *Correlation of constitutive mRNA expression of genes identified by standard compare analysis with IC_{50} values for shikonin of 60 NCI cell lines.*

Symbol	Genbank	Compare coefficient	Name	Function
Standard Compare:				
GIT2	D63482	0.750	G protein-coupled receptor kinase interactor 2	GTPase activator
RPL9	U09953	0.716	Ribosomal protein L9	structural constituent of ribosome
WAS	U12707	0.711	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)	small GTPase regulator
not specified	AC004537	0.709	unknown	unknown
SPN	J04168	0.702	Sialophorin (gpL115, leukosialin, CD43)	transmembrane receptor
RPL34	L38941	0.696	Ribosomal protein L34	structural constituent of ribosome
DOCK2	D86964	0.695	Dedicator of cytokinesis 2	guanine nucelotide exchange factor, T-cell receptor binding
ZNFN1A1	AI247840	0.694	Zinc finger protein, subfamily 1A, 1 (Ikaros)	DNA binding, transcriptional regulation
BLM	U39817	0.693	Bloom syndrome	ATP-dependent DNA helicase
HCLS1	X16663	0.691	Hematopoietic cell-specific Lyn substrate 1	transcription factor
MYB	M15024	0.688	V-myb myeloblastosis viral oncogene homolog (avian)	transcriptional activator
not specified	U22376	0.687	unknown	unknown
NACA	AF054187	0.686	Nascent-polypeptide-associated complex α polypeptide	transcriptional regulation, protein biosynthesis and transport
RPL5	U14966	0.686	Ribosomal protein L5	structural constituent of ribosome
HCLS1	X16663	0.684	Hematopoietic cell-specific Lyn substrate 1	transcription factor
WAS	AI655719	0.684	Wiskott-Aldrich syndrome (eczema-thrombocytopenia)	small GTPase regulator
DOCK2	D86964	0.684	Dedicator of cytokinesis 2	guanine nucelotide exchange factor, T-cell receptor binding
LCP1	J02923	0.683	Lymphocyte cytosolic protein 1 (L-plastin)	actin binding, overexpressed in tumorigenesis
not specified	U25789	0.683	Unknown	unknown
GFI1	U67369	0.682	Growth factor independent 1	transcription factor, tumor progression
RPS12	AA977163	0.678	Similar to ribosomal protein S12	structural constituent of ribosome
RPL15A	W52024	0.678	Ribosomal protein S15a	structural constituent of ribosome
PTPN7	D11327	0.677	Protein tyrosine phosphatase, non-receptor type 7	protein tyrosine phosphatase
CORO1A	D44497	0.677	Coronin, actin binding protein, 1A	structural component of cytoskeleton
not specified	X79234	0.676	Unknown	unknown
Reverse Compare:				
ANXA2	D00017	0.726	Annexin A2	cytoskeleton- and membrane-binding protein exocytosis
ANXA2P3	M62895	0.700	Annexin A2 pseudogene 3	unknown
ITGB5	N29501	0.698	Integrin, β 5	Fibronectin receptor
TEAD1	H96798	0.677	TEA domain family member 1 (SV40 transcriptional enhancer factor)	transcription factor
RBM9	AA025590	0.676	RNA binding motif protein 9	transcriptional activator and corepressor
not specified	R05471	0.670	Unknown	unknown
not specified	W15410	0.652	Unknown	unknown
PROCR	AA039570	0.651	Protein C receptor, endothelial (EPCR)	receptor activity
not specified	AA046660	0.650	Unknown	unknown
GNA11	N36926	0.650	Guanine nucleotide binding protein (G protein), α 11 (Gq class)	signal transducer
GNG12	AA047421	0.649	Guanine nucleotide binding protein (G protein), γ 12	signal transducer
GNA11	N36926	0.645	Guanine nucleotide binding protein (G protein), α 11 (Gq class)	signal transducer
SEPT10	AA044396	0.644	Septin 10	GTP binding, cytokinesis
ANXA2	AA057534	0.642	Annexin A2	cytoskeleton- and membrane-binding protein exocytosis
NCKAP1	AI979116	0.637	NCK-associated protein 1	protein binding
LAMB1	AA004918	0.634	Laminin, β 1	extracellular matrix structural constituent
not specified	T57061	0.631	Unknown	unknown

Table III. *continued*

Table III. *continued*

Symbol	Genbank	Compare coefficient	Name	Function
LOC51035	AA010706	0.630	Unknown	unknown
ARHE	AA037493	0.626	Rho family GTPase 3	GTP binding
not specified	R40626	0.623	Unknown	unknown
not specified	R40626	0.616	Unknown	unknown
SH3BP4	W72796	0.615	SH3-domain binding protein 4	signal transducer
TBC1D8	W69438	0.614	TBC1 domain family, member 8 (with GRAM domain)	GTPase activator
RAI14	AA034024	0.614	Retinoic Acid induced 14	unknown
not specified	W74616	0.612	Unknown	unknown

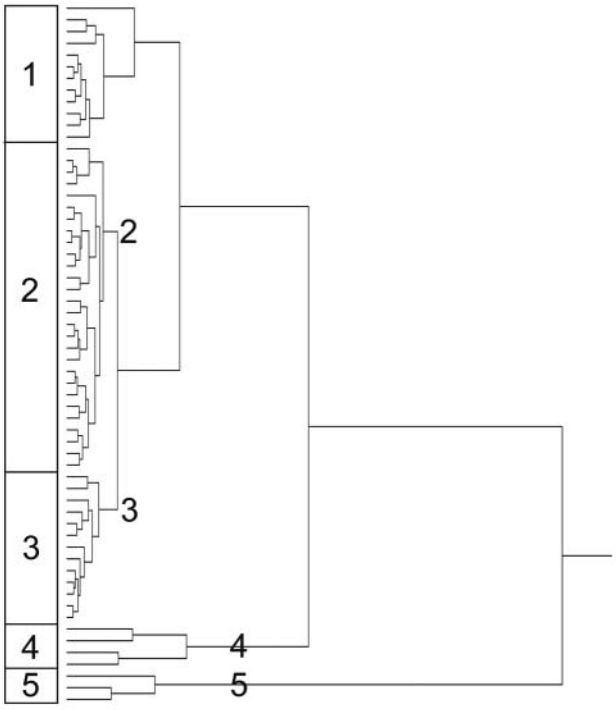

Figure 4. *Dendrogram of hierarchical cluster analysis (complete linkage method) obtained from mRNA expression of genes correlating with $log_{10}IC_{50}$ values for shikinon. The dendrogram shows the clustering of 60 cell lines of the NCI's screening panel according to the mRNA expression profile for 50 genes identified by COMPARE analysis (Table III).*

natural products capable of inhibiting these targets. This may represent the basis for the development of rational treatment of diseases such as cancer. This kind of research also opens avenues for the prediction of the response of individual cancer patients to therapy. We expect that strategies for individualized tumour therapies will lead to improved results for the patients. Molecular-targeted small molecule inhibitors have the potential of increasing tumour

Table IV. *Separation of clusters of 60 NCI cell lines obtained by hierarchical cluster analysis shown in Figure 2 in comparison to drug sensitivity. The median $log_{10}IC_{50}$ value (M) for each compound was used as a cut-off to separate tumor cell lines as being "sensitive" or "resistant".*

	Shikonin		Cycloshikonin	
	sensitive (<–5.829)	resistant (≥–5.829)	sensitive (<–5.6805)	resistant (>–5.6805)
Cluster 1	8	4	8	4
Cluster 2	13	14	15	13
Cluster 3	1	12	1	12
Cluster 4+5	7	0	6	1
χ^2-test (*p*-value)	0.00052		0.00245	

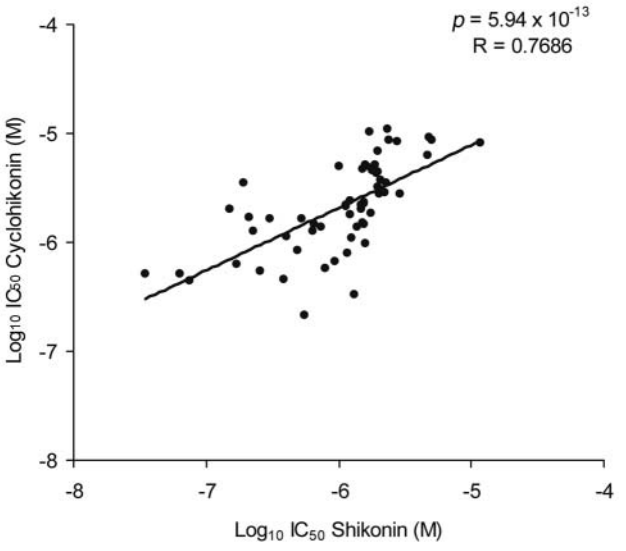

Figure 5. *Correlation of $log_{10}IC_{50}$ values for shikonin and cycloshikonin in the NCI panel of tumour cell lines.*

specificity and reducing adverse side-effects on normal tissues. This concept of individualized tumour therapy is also of great importance for small molecule inhibitors derived from Kampo medicines and other traditional herbal medicines as conceptually shown by us in the past (31-46).

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