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Application of the "-Omic-" technologies in phytomedicine

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Abstract

The proof of efficacy of phytopreparations and the determination of their mode of action are permanent challenges for an evidence-based phytotherapy. The technology platform of genomics, proteomics and metabolomics ("-omic-" technologies) are high-throughput technologies. They increase substantially the number of proteins/genes that can be detected simultaneously and have the potential to relate complex mixtures to complex effects in the form of gene/protein expression profiles. Provided that phytopreparation-specific signatures in the form of gene/protein expression profiles can be developed, these technologies will be useful for the chemical and pharmacological standardization and the proof of the toxicological potential of a plant extract. Over a long-term perspective they may economize the proof of efficacy, the determination of the mode of action of phytomedicines and allow to investigate herbal extracts without prominent active principle(s). The application of this genomics revealed already that gene expression profiles induced by single drugs and the ones induced by the combination of the same drugs can be entirely different. These results make the information of the mode of action of isolated "active principles/lead substances" of phytopreparations questionable. The application of the "-omic-" technologies may lead to a change of paradigms towards the application of complex mixtures in medicine and open the new field of phytogenomics, -proteomics and -metabolomics.

Keywords: Phytomedicine; Genomics; Proteomics; Metabolomics; Synergy

Introduction

The recent technical developments in genomics, proteomics and metabolomics have created great excitement and optimism in the field of life science research. Genomics aims at the comprehensive description of the genetic information, proteomics at the description of proteins and metabolomics at the qualitative and quantitative analysis of all low-molecular-weight metabolites of a cell or organism and their dynamics in biological systems. These

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new platforms of the so-called-"-omic" technologies allow the analysis and characterization of biological systems in so far unknown details - tens of thousands of genes and proteins can be detected simultaneously. This represents a challenging complexity for scientific analysis and will open new perspectives for ethnobotanical and phytomedical research purposes. Before highlighting expected changes in plant research, it will be useful to briefly describe the technical basis for these "-omic" technologies.

Genomics and transcriptomics

The definition of genomics is not precise. The term wasn coined by Tom Roderick and originally meant analysis of the whole genome. Now it commonly refers to largescale, high-throuput molecular analysis of multiple genes, gene products or regions of genes (Cook-Deegan *et al.*, 2000). Transcriptomics, often included in the term genomics, depicts the expression level of genes. For both, the new tools are the microarrays. The term "micro-array" itself, often called "biochip", simply describes that a high number of molecules (oligonucleotides) are arranged on an extremely small space, commonly on a glass surface (up to 200.000 spots/cm^2) (Fig. 1). The interactions of RNA- or DNA extracts with these biochips are investigated and allow a simultaneous analysis of pleiotropic alterations at the genome and transcriptome level. Based on the target sequences on the glass surface, hundreds of genes can be targeted and significant changes of their mRNA can be estimated simultaneously. Identified functional gene clusters (Fig. 2) with uniform change in expression levels allow predictions of major changes in e.g. metabolic pathways leading over to the next "omic" - technology - proteomics.

Fig. 1. Picoliterprobes are spotted on glass carriers. The spot density can vary (3000 - 10,000 spots/cm^2) and up to 20,0000 spots are brought on one glass carrier. Spots are organized in grids and supergrids. The bare code at the bottom allows the correct identification. Kindly provided by Miltenyi Biotec.

Fig. 2. Cluster Analysis groups relevant genes according to their similar expression profiles. All genes are ordered in a hierarchical tree. The shown cluster shows correlated groups of genes and experiments. Neighboring genes or experiments in the tree are similar, distant ones are different from each other. For example gene 9 is induced in experiments 1, 3 and 5, repressed in experiments 2, 4 and 6. It is most similar to gene 11. Red: induction; green: repression; black: no differential regulation. Kindly provided by Miltenyi Biotec.

Proteomics

The term proteome was proposed by Wilkins *et al.*, (1995, 1996) and depicts the entire PROTEin complement expressed by a genOME. Proteomics is the largescale study of proteins, particularly their structures and functions. The term was coined to create an analogy with genomics. Although it is often viewed as the "next step", proteomics is more complicated than genomics. The Human Genome Project revealed that there are fewer protein-coding genes in the human genome than there are proteins in the human proteome (~22,000 genes versus ~400,000 proteins). This discrepancy implies that protein diversity cannot be fully characterized by gene expression analysis alone, making proteomics a useful tool for characterizing cells and tissues of interest. The key technologies used in proteomics are one- and two-dimensional gel electrophoresis to identify the relative mass of a protein and its isoelectric point (Williams *et al.*, 2003). Affinity chromatography, fluorescence resonance energy transfer and surface plasmon resonance are used to identify protein-protein or protein-DNA interactions. X-ray tomography is used to determine the location of proteins or protein complexes in labeled cells. Further, fluorescent proteins like green fluorescent protein (GFP), yellow FP, cyan FP or red FP are frequently used to study cellular events such as localization of proteins to membranes and to cellular organelles. They can mark homogenous populations of specialized cells whose gene expression profiles should be determined by DNA micro array analysis, In fact, FPs like GFP are often used as direct transcriptional and translational reporters in living cells in a way linking transcriptomics and proteomics.

Metabolomics [1]

Metabolic analysis can be divided into four areas: (1) target compound analysis - the quantification of specific metabolites, (2) the metabolic profiling-the quantitative and qualitative determination of a group of related compounds or of specific metabolic pathways, (3) metabolomic - the qualitative and quantitative analysis orall metabolites and (4) metabolomic fingerprinting -sample classification by rapid global analysis.

[1]Definitions: *Metabolic/ metabolite profile*: determination of metabolites of an organism without the demand of a complete analysis. Commonly certain groups of metabolits like lipids or organic acids are measured/. Sometimes used as synonym for metabolornics. *Metabolome*: the totality of small molecules. which are formed by a cell, tissue or organism under certain conditions, and under consideration of the concentration. *Metabolomics*: term was coined in the plant research. It has the demand to determined the metabolom quantitatively and completely. *Metabonomic*: term was coined in the field medicine/pharmacology and is primarily used here. It describes the measurement of the metabolic reaction towards medication, environment or diseases, It .is comonly substituted by the term metabolomics.

The techniques used are multidisciplinary: for target compound analysis and metabolic profiling, the main techniques are gas chromatography, high-performance liquid chromatography and nuclear magnetic resonance (NMR). Further, metabolomics makes use of several complementary analytic methods; in particular, "hyphenated" techniques of LC/MS, LC/MS and LC/NMR are likely to have increased impact. A more detailed description of each method is given in Metabolomics - nrp (2006). These approaches rely on chromatographic separations, often coupled with well-developed calibrations for specific analytes. The metabolic fingerprinting analyses crude extracts without any separations step, using NMR, direct injection mass spectrometry (MS) or Fourier transform infrared spectroscopy.

Systems biology

The combined information from genomics, proteomics and metabolomics will help us to obtain an integrated understanding of a cell or organism. However, these new analytic platforms are high-throughput technologies which substantially increase the dynamic range and number of metabolites and genes that can be detected (Kell, 2006; Dunn and Ellis, 2005; Kell and Mendes, 2000). This has created an increasing need for informatic tools to transform parallel information into real biological data and knowledge (Wang *et al.*, 2005a). One outcome of the development of informatic tools is the advancement of systems biology. In systems biology, especially metabolomic data are presently organized with the aim to create computer models simulating biological system. Since the metabolic control analysis and functional genomics share the same agenda (Kell and Mendes, 2000), systems biology is expected on the long term to predict both genomic activations and metabolite flows in complex systems. Their joint application is already now judged to be the ultimate phenotyping of a cell or plant and considered to have the potential to revolutionize natural product research and to advance the development of scientific based herbal medicine (Wang *et al.*, 2005a; Verpoorte, 2005; Patwardhan *et al.*, 2005b). For example these technologies are likely to change and expedite the toxicological profiling of plants or drugs. In addition, the integration of these data into systems biology is expected to enable the study and understanding of living systems from a holistic perspective and to become the adequate tool to analyze complex traditional systems of medicine (TSM).

Therapeutic approaches: "herbal shotgun versus silver bullet"

Phytobotanical and ethnobotanical research have focused for decades on the search for the mostly single "active principle" in plants, based on the assumption that a plant has one or a few ingredients which determine its therapeutic effect. However, European phytomedicine and traditional Asian systems of medicine like the TCM and Ayurveda generally assume synergy to be of vital importance with the application of. phytomedicine (Williamson, 2001). To complicate matters further, herbalists use preparations and mixtures which not necessarily intend to target a particular organ, cell tissue or biochemical systems (Williamson, 2001). This kind of application has been described as the "herbal shotgun" approach, as opposed to the "silver bullet" method of conventional medicine (Ducke and Bogenschutz-Godwin, 1999; Williamson, 2001) to distinguish the multitargeted approach of herbals from the mono target approach of synthetic drugs addressing specific enzymes or receptors.

This "shotgun approach" has so far been the major complication of phytomedicine as summarized in a quotation of MacKenzie (2001) and Williamson (2001) *"To market herbal derivath;es with full patent protection, there would have to be (they would have to do) clinical trials on the active ingredients, separately and together. Compared with' testing a single magic bullet, this is prohibitively expensive" and "without the support of the pharmaceuticals industry, herbs are likely to remain mired in uncertainty. What a waste."*

In recent years this "shotgun approach", understood as a therapeutic strategy which aims at multiple targets in an organism, has gained an increasing acceptance. We even experience a paradigm shift (Wagner, 2001) caused by the growing evidence of the multifactorial nature of today's disease

challenges. Evidences increase that the side effects of a combination therapy are not necessarily additive, but may be even less than the ones of the single therapy and combined with a higher therapeutic efficacy. Well-established combination therapies are e.g. the cancer chemotherapies, or the treatment of HIV and hypertension. The combination of methotrexate (MTX) and TNF-α antagonists in rheumatoid arthritis was recently shown to be more efficient compared to single applications (Geletka and St.Clair, 2005) while having less or not more than equal side effects. We have recently demonstrated that the application of a multimodal therapeutic strategy can successfully cure the autoimmune disease acquired hemophilia (Zeitler *et al.*, 2005). In the context of systems biology, Kell (2006) demonstrated in a model analysis how targeting a particular step in the signaling pathway of the transcription factor NFB can have qualitatively (directionally) different effects depending on the actual state of the system. For some values of the two rate constants of the reaction, there was no influence on the signaling pathway. Other combinations could lead to entirely opposite effects. This type of systemic nonlinearity of biological systems makes designing safe drugs a challenging activity and can also account for the unexpected synergetic effects often observed when different metabolic pathways or drug targets are affected at the same time, both in theory (Cornish-Bowden *et al.*, 1995; Fell and Thomas, 1995; Fell, 1998, Cascante *et al.*, 2002) and in practice (McCafferty *et a1.*, 1999; Fan *et al.*, 2003; Borisy *et al.*, 2003; Kel1, 2006). Ke1l, (2006) and Verpoorte e*t a1.* (2005) even estimate that these results of systems biology will lead to a replacement of the single compound or single target approach by multitarget approaches.

But even though multitarget treatment approaches are generally more accepted, phytopharmaceuticals still need to proof efficacy, provide a rational behind this efficacy and demonstrate reproducibility through standardization. The standardization of the herbal extracts safeguards the pharmaceutical quality and forms the prerequisite for the reproducibility of the effect from batch to batch (Saller and Reichling, 2002). But phytopharmaceuticals are biogenic products and can thus have fluctuating compositions of ingredients. According to the region or the harvest, season efficacy and toxicity of the crude extracts may vary. In spite of this somewhat "undefined" chemical state, phytochemicals have been used as therapeutic agents based on the chemical characterization of single ingredients which so far have been supposed to determine their mode of action (Saller and Reichling, 2002). In addition, the German (AMG[3] § 26, paragraph 2) and the European law (Benedum, 1998) allow under certain circumstances in accordance with the WHO guidelines, that written historical material, if assessed with scientific methods, can be used as valid scientific source material or substitute for a proof of efficacy. These regulations take into consideration that many plants have a centuries-old traditional usage in their countries of origin. Nevertheless, in order to comply with the legal conditions with respect to effectiveness, quality and safety, certain steps of standardization are mandatory in Europe (Table I). A detailed review is available in Wagner (2001). The standardization includes the correct taxonomic identification of the plant(s) and the identification of the major active principle(s) of plants, extract(s) or multiextract mixtures. The identification of the active principle(s) is followed by purification and concentration. The "unwanted" effects of accompanying plant ingredients are eliminated and the effect through concentration of the isolated component(s) is

Table 1. Quality assurance of phytopreparations

1	Definitive authentication and taxonomic assignment e.g. through DNA-fingerprinting + DNA barcoding
2	Isolation and structural elucidation of all major constituents of the herbal drug
3	Identification of the true bioactive constituents
4	Multiextract mixtures: standardization of the single extracts or 3D HPLC fingerprint analysis of the multicompound extracts
5	Global harmonization of standardization criteria under the umbrella of the International Federation of Pharmaceutical Manufacturers Associations (IFPMA)

increased. In case of drug development, a chemical synthesis of the identified single active principle may follow. The single isolated chemically defined component(s) or its synthesized derivate(s) are not termed phytopharmaceuticals any more (Saller and Reichling, 2002). As examples among the newest isolated and therapeutically used compounds only taxol, camptothecine and artemisinin are mentioned.

Polyvalent pharmacological activity and synergy

Many phytopharmaceuticals on the today's drug market are crude extracts and thus complex mixtures of compounds. Detailed pharmacological experiments with single isolated compounds versus the original extract or extract fractions have confirmed that many plant constituents, among them primarily phenolic compounds and terpenoids, exert polyvalent pharmacological effects (Wagner, 2001). This might explain some of the pharmacological synergetic effects and the phenomenon that very often an extract possesses a much better therapeutic effect than single isolated constituents. Examples for polyvalent pharmacological activities and proven synergistic effects are given in Table 2. Hawthorn (*Crataegus oxyacantha*) e.g. is indicated for the treatment of heart insufficiency grades I and II (NYHA I u. II). It was shown to posses a positive inotrop activity and an ACE-inhibitory and vessel dilatative activity in angina pectoris. The active compounds of *Garlic (Allium sativum)* ajoene and allicine have a cholesterol- and lipid lowering activity, they act as antioxidants, inhibit. NO formation and have an anti-hypertensive action. It was also shown that ajoene induces apoptosis in human leukemic cells probably via the induction of reactive oxygen species; however the clinical relevance of this has not yet been investigated (Wagner, 2001; Dirsch *et al*., 1998a, b). These examples demonstrate that active compounds of a plant can act via different mechanisms of actions with a relevance to entirely different diseases. The well-documented efficacy of the root of *Urtica dioica* in prostate hyperplasia is probably based on a synergism of anti proliferative and antiinflammatory effects caused by lectins and polysaccharides. Interestingly also the leaves of *U. dioica* possess antiinflammatory effects due to the content of oxylipines (13-hydroxy-octadecatrienoic acid) and caffeic acid derivatives. They are used in the treatment of arthritis (Klingelhofer, 2001; Obertreis *et al*., 1996). Another classical example is the "Salix extract l520L" which inhibits cyclooxygenase-2-mediated prostaglandin E2 release through compounds other than salicin or salicylate (Fiebich and Chrubasik, 2004). In the case of *Harpagophytum procumbens* much higher concentrations of the "active principle" harpagosid are necessary for the downregulation of iNOS expression in rat mesangial cells than available in an effective concentration of the total extracts (Kaszkin

et al., 2004). Further, an activity-directed fractionation of the herbal extract revealed a wide distribution of the desired activities in the plant with no clear separation into polar and non-polar fractions (Gilani *et al.*, 2005). Another classical example is *St. John's worth (Hypericum perforatum)*, in which no single active compound has been found that can explain the full proven clnical activity (Mueller, 2005; Verpoorte *et al.*, 2005).

In these cases, the application of the refined herbal extract rather than "isolated active principle(s)" may be favored in order to benefit from the broad therapeutical and pharmacological action related to the special composition of the ingredients in the entire plant. Clinical and experimental evidences on synergy are reviewed in Williamson (2001). They include the plants *Ginkgo biloba, Piper methysticum, Glycyrrhiza glabra, Cannabis sativa*, flavonoids and essential oils or mixed herb formulations as the Indian "Trikatu" or the combination of *Urtica dioica* and *Pygeum africanum* (Williamson, 2001).

The presence of synergistic effects has consequences for the determination of the active principle(s) of plant extracts as demanded in Table 1. Williamson has summarized this dilemma in the following quotation: *"If a combination of substances is needed for the effect, then the bioassay-led method of investigation, narrowing activity down firstly to a fraction and eventually a compound is doomed to failure and this has led to the suggestion that the plants are in fact devoid of activity"*.

Evidences from the "-transcriptomic" technology

Can the application of the transcriptomic technology support the identification of synergy and polyvalent pharmacological activities?

Recently, micro arrays were applied for the investigation of the combination therapy of MTX and mercaptopurin. It was demonstrated that the combination therapy of both to treat human leukemia cells causes a different gene expression profile than the single application of each drug. Only 14% of genes that changed when these medications were given as single agents also changed when they were given in combination. Thus, 86% of the previous upregulated genes did not respond. Discriminating genes that changed when these medications were given together included those involved in apoptosis, mismatch repair, cell cycle control and stress response (Cheok *et al.*, 2003).

These findings demonstrate that complex gene expression analysis by micro array can detect differences in cellular responses to drug combinations versus single agents (Cheok *et al.*, 2003). It further shows that drug combinations can lead to the activation of entirely different genes than those genes activated by the individual single agents. Thus, the mode of action of the combination is, based on the gene expression, entirely different from the mode of action of the single agents. Although one may question whether the discriminating genes for the treatments are the same which are responsible for the main action of the single agent or not, the authors conclude that the method is highly suitable for the discrimination of different treatments (Cheok *et al.*, 2003). Similarly Schulte *et al.*, (2003) demonstrated that the combined treatment of neuroblastoma cells with cisplatin and hyperthermia lead to the upregulation of 131 new genes which were not expressed under treatment with either cisplatin or hyperthermia alone, confirming that multimodal treatment approaches can apparently led to different effects on the level of gene expression.

Table 2. Herbal drugs with evidences for synergistic effect/polyvalent activities

Herb/major constituents	Effects/indications
Allium sativum [a,b,c]	
Allicin + Ajoene	Inhibition-inducible nitric oxide synthase expression in activated macrophages, inhibition of thrombocyte aggregation, antiinflammatory, triglyceride- and cholesterol-decreasing, antioxidant, antimicrobial
Ajoene	Induction of apoptosis in human leucemic cells, production of intracellular peroxides
Cannabis sativa [d,e,f]	
Tetrahydrocannabinol	Muscle-relaxant, appetite-stimulating, analgesic effects
Cannabidiol	Amplification of the antispastic activity
Crataegus oxyacantha [c,g]	
Procyanidines, flavon-C-glycosides	Cardiotonic activity, angiotensin-converting enzyme inhibiting effect, endothelin-dependent, smooth muscle-relaxing effect
Ginkgo biloba [h,i]	
Ginkgolides A + B	Synergy effects in a thrombocyte-aggregation inhibiting assay
Glycyrrhiza glabra [j,k,l]	
Glycyrrhizin/isoliquiritin	Antitussiva, anti-inflammatory/amplification of activity of hydrocortisone
Harpagophytum procumbens (Burch); *H. zeyheri (Decne)* [c,m]	
Harpagosid + components of total extract	Stronger inhibition of leucotriene-and thromboxane-biosynthesis than harpagoside alone
Hypericum (St, John's wort) [n,o]	
Hypericins, hyperforin, xanthones, flavonoids, procyanidins	Antidepressiva, no prominent lead substance
Piper methysticum [p,q]	
Yangonin, desmethoxy-yangonin dihydromethysticin	Anxiolytic, sedative, anti-convulsant, spasmolytic, anti-inflammatory, analgetic activity
Salix cortex L. [r,s,t]	
Salicin + salicylalcohol derivates, flavonoids, tannins	Stronger antiinflammatory and analgesic effect than salicin alone
Urtica dioica L, (radix) [h,u,v]	
Urtica dioica agglutinine (UDA) + Polysaccharides	Competitive inhibition of EGF induced proliferation lmmunostimulation
Urtica dioica L. (folium)	
Oxylipine (13-Hydroxyoctadecatrienoic acid), caffeic acid derivatives	Cyclooxygenase- and cytokine inhibition for the treatment of arthritis and rheumatoid arthritis
Valeriana officinalis [w]	
Valtrate, isovaltrate,valerenone, valerenic acid	Sedation, increasing GABA concentrations
Viscum album [x,y]	
Mistletoe lectin I	Induction of apoptosis of cancer cells, immunostimulating activity
Zingiber officinale (essential oil) [c,z]	
α-Zingiberene, β-sesquiphellandrene Bisabolene, curcumene	Whole ginger preparation with different effects than single components. Antipyretic, analgetic, cardiac inotrop, sedative, antibiotic + others

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Role of the "active principle"

Based on the above results, it should be questioned whether it makes sense to focus on a single mode of action of a plant or plant extract by searching and isolating the active principle(s) or whether it may be more meaningful to screen refined herbal extracts for their complex modes of action on a microarray directly. If a combination of constituents yields a new mode of action, separate clinical trials on single active constituents would be superfluous. This would tremendously accelerate and economize phytomedical research on time and work. The development of gene expression

signatures for extracts would allow a fast screening and would simultaneously generate the potential to cover synergistic effects of the plant extracts. However, the development of these expression signatures with the micro array technology is nowadays highly cost intensive with one microarray analysis costing easily 1200 €.

The presently used method to proof synergy is the isobole method (reviewed in Williamson, 2001). This method is independent of the mode of action and measures the (end-) effect. Synergy takes place if the dose-response curve demonstrates that the achievement of a certain effect requires less dosages of two substances than expected from their individual dose response curve. An example of such a dose-response curve is given in Fig. 3 for the ginkgolides A and B as measured in a thrombocyte aggregation inhibiting assay. The method, however, is sill a demonstration of "additivity" or "superadditivity" - related to the effect measured. The micro array results of Cheok *et al*., (2003) bring into synergy an entirely different quality. They demonstrate that the combination of two components brings about a new mode of action resulting possibly also in new effects.

The necessity to look into synergistic effects in phytomedicine may also be underlined by the following figures: the mass bioprospecting effort of the national cancer institute of the United States screened about 114,000 extracts from an estimated 35,000 plant samples against a number of tumor systems (Cragg and Boyd, 1996). A wide variety of compounds with different structures were isolated and characterized (Soejarto *et al.*, 2005). Clinically significant cancer chemotherapeutic agents that emerged from this project included for example paclitaxel (Taxol®), topotecan (Hycamtin®) and CPT-11 (Taxman *et al*., 2003). The latter two compounds are semisynthetic derivates of camptothecin from

Camptotheca acuminata Decne., Nyssaceae (Soejarto *et al.*, 2005). The yield of these huge screening projects for the identification of single bioactive compounds appears moderate. A provision for synergistic effects might elevate the yield.

Recent applications of DNA microarrays and metabolomics to phytomedicine

Microarray technology has so far not been used extensively in phytomedicine and is presently in the stage of "proof of principle". Wang *et al.*, examined the effect of so-called "herbal glycoside recipes" on the ability of spatial learning memory in mice suffering from cerebral ischemia/reperfusion. The herbal preparations were obviously derived from the roots of *Scutellaria baicalensis* and *Dioscorea spp.* and contained baicalein (5, 6, 7-trihydroxyflavone) and dioscin (ratio 1:1). Using a cDNA micro array system containing 1176 known genes, Wang *et al*., (2004) showed a reproducible dose-dependent effect of these herbal preparations and suggested the usefulness of this methodology for elucidating the mechanism of pharmacological functions of herbal preparations.

A very recent report describes the analysis of two soja bean extracts. The gene expression profiles of the herbal extracts were compared with those of the single phytoestrogens. The profiles of the extracts correlated with those of the phytoestrogens, but gave quite different *R*-values for each phytoestrogen (Ise *et al.*, 2005). Interestingly, the gene expression profiles induced by 10 mM of the phytoestrogen daidzein correlated with those derived from the total extracts (*R*-values:0.73 and 0.75), but the estimated concentrations of daidzein in the extracts were much lower. They were roughly 1/100 of 10 mM (Ise *et al.*, 2005). Mur *et al.*, (2006) investigated the interaction of plant components. Salicylic acid has been proposed to antagonize jasmonic acid biosynthesis and signaling in plants. Microarray analysis demonstrated that the combination of both acted transiently synergistic on certain gene expressions (defensin and thionin, beta-glucuronidase) in tobacco plants when both were applied at low concentrations, but antagonism was observed at more prolonged treatment durations or at higher concentrations. The authors concluded that there seems to be a greater sophistication in interactions than "simple" antagonism or synergism (Mur *et al*., 2006). Instead, synergistic/antagonistic mechanisms may represent positive and negative feedback loops of the same molecule combination allowing the tailoring of the plant response to a particular situation.

First clinical studies in the application of a metabonic strategy, utilizing high-resolution ^{1}H NMR in conjunction with chemometric methods showed that a clear differentiation of metabolite profiles before and after *Chamomile* tea drinking can be obtained although strong extrinsic physiological variations were observed. About 14 volunteers had ingested *chamomile* tea for a period of 2 weeks. Urine samples before, during and after *chamomile* ingestion were analyzed. *Chamomile* tea ingestion was shown to lead to an increased urinary excretion of hippurate and glycine with depleted creatinine concentrations. This study highlights the potential for the metabonic technology in the assessment of "small" interventions despite a high degree of variation from genetic and environmental sources (Wang *et al.*, 2005b). Variations in diet or local environment can become important confounding factors when metabolic responses to nutritional or minor interventions are studied. To extract meaningful biological information from data confounded by such diverse extraneous physiological variations, data-filtering methods can be employed. One of the most frequently used data-filtering

methods is orthogonal signal correction (OSC) which was also applied in the above study.

In the field of molecular toxicology, the high-quality gene arrays commercially available have already allowed this technology to become a standard tool (Lettieri, 2006). Several national and international initiatives provided the proof-of principle tests for the application of gene expression for the study of toxicity and new existing chemical compounds (Lettieri, 2006). In the United States, the national institute of environmental health science has created the national center for toxicogenomics to provide a reference system of genome-wide gene expression data and to develop a knowledge base of chemical effects in biological systems (Tennant, 2002; Lettieri, 2006). Studies here showed that it is possible to identify a signature of expressed gene patterns after exposure to a given toxicant (Tennant, 2002; Lettieri, 2006).

These reports are promising for the application of micro arrays in phytoresearch and phytomedicine and they are likely to change or develop our understanding of synergy. However, the standardization of herbal extracts remains crucial. It may be simplified using those plant ingredients which show the maximum overlap in gene expression with the refined extract as shown in the example of the soja extract and daidzein (Ise *et al.*, 2005). Also, Verpoorte has already hypothesized that by measuring the activity in a living organism for extracts with different composition, one may possibly identify a compound or a combination of compounds that correlate with the activity. This means that activity due to synergism and also activity of pro-drugs can be recognized (Verpoorte *et al.*, 2005).

Proprietary drugs in the - "omic - technology" - Aera

The so-called "proprietary drugs" prescribed in the European, Chinese and Indian systems of medicine have for long been used in the West as source material for the development of new drugs. The potential of fixed combination formulae which are mostly applied in the TSM could so far not be exploited. Their application is often already described with exact time tables and antidots. For example Ayurveda describes to counteract the "side effect" of *Semicarpus anacardium* preparations with Fructus Coriandri (Ulrich-Merzenich, .1998). In addition, dosages during summer seasons are to be reduced, with higher dosages necessary during winter seasons. These peculiar knowledge could so far not be explored. The "omic-technologies" are tools, even though costly and laborious, which are likely to provide a basis to investigate these complex phenomena and subsequently substantiate or dismiss such recommendations.

A few drug formulations ofTSM have recently gained interest - the ayurvedic formulation "Trikatu" or the Chinese medicine "Iijen". Further details and additional examples are given in Table 3. More than half of those herbal preparations were tested against synthetic standard substances in clinical placebo-controlled doubleblind studies. The fact that they have shown at minimum a therapeutic equivalence with often less or no side effects - results not necessarily to be expected - is another indicator for [the likelihood of] synergistic effects of phytopreparations.

Patwardhan (2005a) and Patwardhan *et al.* (2005b) reviewed citation and patent data for Indian and Chinese medicine. The Pubmed search revealed 1045 citations for Indian and 10,278 citations for Chinese medicine; so far 3 US patents have been granted for ayurvedic medicines and 195 for Chinese medicines (Patwardhan *et al.*, 2005b). These figures demonstrate the growing interest in fixed combinations, but also the already-mentioned dilemma of patenting complex mixtures as force to develop phytomedicine. For patenting, novelty

Table 3. Drugs from traditional medical systems of Asia, China and Europe intensively investigated

Antipsoriatic composition	*Argemone mexicanae* (US-patent application)
Arthritis	*Boswellia serrata, Curcuma longa, Withania somnifera, Zingiber officinale (Artrex®, US-Patent); Phytodolor® (Fraxinus excelsior, Populus tremula, Solidago virgaurea)*
Bowl syndrome *(Colitis ulcerosa)*	Ayurmedica H-IS kaps (*Boswellia serrata*)
Gastric and abdominal disorders	*Piper longum, Zingiber officinale* (Trikatu) Iberogast® *(Iberis amara, Angelica archangelica, Matricaria recutita, Carum carvi, Silybum marianum, Melissa officinalis, Mentha piperita, Chelidonium majus, Glycyrrhiza glabra)*
Hyperlipidemia, Atherosclerosis	*Commiphora wightii* (Guglip®, Cipla Ltd)
Non-steroidal anti-inflammatory drug	*Boswellia serrata gum resin* (Sallaki® Gufic)
Non-small lung cancer	*Coix lachryma-jobi* (Phase II Trial US)
General tonic	*Astragalus membranaceus* (Xue baoPG2)
Prevention and therapy of stroke	*Salvia miltiorrhiza, Paeonia rubra, Angelica pubescens, Stephania tetranda. Uncarial C. Uncis, Gastrodia elata, Panax ginseng*
Anticancer drugs	*Camptotheca acuminata*, CPTII, Topotecan ,
Cognitive performance	*Panax ginseng, Ginkgo biloba,* Panax ginseng extract (GK-501) + Ginkgo extract (GK-511)
Insomnia	*Piper methysticum, Valeriana officinalis* (Kava extract (LI-150) + Valerian extract (LI-15))

Summarized from Patwardhan et al. (2005a, b), Schempp et al. (2006), Wagner (2001) and Williamson (2001).

and innovation are required (Verpoorten, 2005). The proof of a unique qualities of herbal medicine and the rationalization of therapeutic effects of complex mixtures of single drugs or of complex mixtures may have consequences on the legislation and patenting. A gene expression signature of an extract represents the documentation of a unique mode of action, presumed that the gene profiles are reproducible. Gene profiles can, however, vary depending on the individual, the age or the dosage. But results of Cheok et al. (2003) and Wang *et al*. (2005b) are promising. Cheok et al. (2003) found treatment-specific gene expression profiles in leukemia cells of 60 individuals for MTX and/or mercaptopurin whereas Wang *et al*. (2005b) demonstrated in a minor intervention treatment-specific changes with *Chamomile* tea. The reproducibility of the new technologies, e.g., gene and protein arrays considering extract variations due to season or batches still needs to be evaluated. Data on these experiments in combination with gene expression profiling in toxicology will decide how easy and how soon we can implement these technologies in the routine standardization process and how far legislation can provide appropriate framework conditions for these new developments.

Reproducibility of microarrays on plant extracts will have consequences also for an often criticized aspect of phytomedicine: So far it is

only accepted that chemically defined compounds cause a defined effect. If it is possible, however, to attribute a reproducible gene expression profile to a chemically not fully defined complex herbal mixture, this paradigm needs to be questioned. Physicists already acknowledged earlier that *"The breakdown of an aggregate as an entity into partial aggregates is, to be precise. not really possible. There is only an entirety which somewhat can be perceived as an entity of parts, but which is then more than the sum of its parts."* (Durr, 1989).

Nevertheless, there are presently still a number of general limitations in the micro array methodology. We have so far not yet identified each and every gene,not to mention their function. Even though a broad spectrum of mRNAs can be determined simultaneously, we are presently still in the process to unravel the association of upregulated mRNA and the protein formation. In addition, the limited knowledge of gene regulation, of the interplay of protein networks and of the large number of regulatory feedback loops makes the interpretation of micorarray- and or/proteome-based data still difficult (Daniel and tom Diek, 2004). For plant extracts, customized or focused DNA microarray and the refinement of the statistical approach by adopting the correlation analysis were proposed to improve the reliability of data (Ise *et al*., 2005). Data filtering methods like the OSC have been proposed and are applied to extract meaningful biological information from data confounded by diverse extraneous physiological variations like age, sex and genetic polymorphisms in natural populations.

Presently, there is a strong demand and necessity to accelerate the research in phytomedicine. The world market for phytopharmaceuticals grows steadily. The estimated turnover in 1995 was US$ 12.4 billion (Benedum, 1998). Today the herbal industry has a turnover of about US$ 62 billion with a strong growth potential (Patwardhan *et al*., 2005b). Sales of herbal supplements including dietary supplements and functional foods increased alone in the USA by 101 % in 1998 within 1 year to a total of US$ 587 million (Benedum, 1998). The World Bank reports state that trade in medicinal plants, botanical drug products and raw materials is growing at an annual growth rate between 5% and 15% (Patwardhan *et al*., 2005b; WHO, 2002). At the same time, Pieters and Vlietinick (2002) resume in a very recent overview about drug development from traditional medical systems that nature's biodiversity has so far remained largely unexplored.

In summary, the presented "omic" technologies allow the simultaneous analysis of complex chains of action and have the potential to relate complex mixtures to complex effects on the different levels of metabolism. The link of reproducible gene/protein expression profiles to phytopreparations will support the development of a causality-based phytotherapy. Even though the assessment of the efficacy of phytopreparations by placebocontrolled trials with bioavailability and pharmacokinetics holds the key for a rational and fully accepted phytotherapy; the application of "omic" technology unfolds the possibility to investigate phytopreparations without prominent active principle(s) for their complex mechanisms of action and helps us to rationalize the therapeutic superiority of many plant extracts over single isolated constituents. Since the mode of action of a drug combination can differ substantially from the mode of action of the same drugs applied individually, the thrust for the search of ttle single active principle may lose its importance, thereby simplifying and economizing the research. Phytomedicine may

become a new challenge towards understanding the effects of complex mixtures on molecular and biochemical processes in health and disease and open up the new field of phytogenomics, -proteomics and -metabolomics. The real age of phytomedicine is yet to come.

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