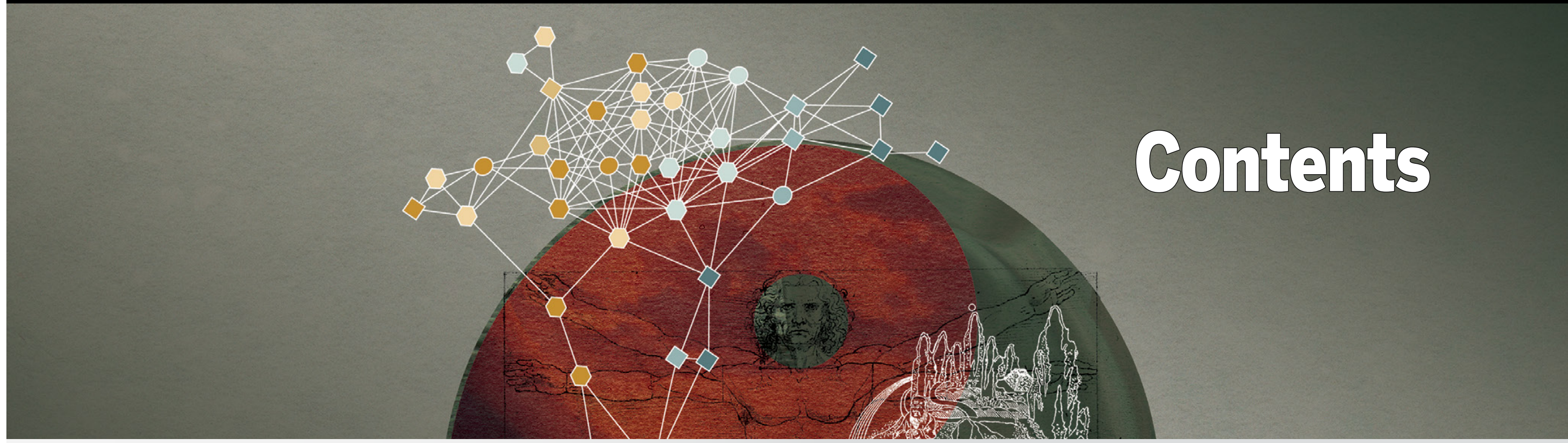
Produced by the *Science*/AAAS Custom Publishing Office

The Art and Science of Traditional Medicine
Part 1: TCM Today – A Case for Integration

Sponsored by

北京中医药大学
BEIJING UNIVERSITY OF CHINESE MEDICINE

香港浸會大學
HONG KONG BAPTIST UNIVERSITY



Contents

In this first installment of a three part series, "The Art and Science of Traditional Medicine," we present a series of articles making a case for the integration of traditional Chinese medicine (TCM) into modern medical practice. From the new WHO Traditional Medicine Strategy to the application of systems biology in studying TCM, we aim to highlight the potential for creating an integrated, network-based health care system. The next two issues will cover herbal genomics and highlight the importance of quality control, standardization, regulation, and safety for traditional therapies. An overview of indigenous medicines in Europe, Africa, the Middle East, India, and the Americas will also be provided.

Editorial Team

Tai-Ping Fan, Ph.D. (Guest project editor)
University of Cambridge, UK

Josephine Briggs, M.D.
National Center for Complementary & Alternative Medicine, NIH, USA

Liang Liu, M.D., Ph.D.
Macau University of Science & Technology, Macau SAR, China

Aiping Lu, M.D., Ph.D.
Hong Kong Baptist University, Hong Kong SAR, China

Jan van der Greef, Ph.D.
University of Leiden and TNO, The Netherlands

Anlong Xu, Ph.D.
Beijing University of Chinese Medicine, China

Editor: Sean Sanders, Ph.D.
Assistant Editor: Tianna Hicklin, Ph.D.
Proofreader/Copyeditor: Yuse Lajiminmuhip
Designer: Amy Hardcastle

Bill Moran, Global Director
Custom Publishing
bmoran@aaas.org
+1-202-326-6438

Ruolei Wu, Associate Director, Asia
Custom Publishing
rwu@aaas.org
+86-186-0082-9345

ILLUSTRATION (FRONT) CHARLOTTE LOKIN

Supporting the integration and modernization of traditional medicine

Nearly a quarter of all modern medicines are derived from natural products, many of which were first used in a traditional medicine context.

Traditional medicine (TM) holds great potential to improve people's health and wellness. It is an important, yet often underestimated, part of health care. TM is found in almost every country in the world and the demand for its services is increasing every day. TM can contribute to addressing a number of global health challenges of the 21st Century, in particular in the area of chronic, noncommunicable diseases and population aging.

TM is often seen as more accessible, more affordable, and more acceptable to people and can therefore also represent a tool to help achieve universal health coverage. It is commonly used in large parts of Africa, Asia, and Latin America. For many millions of people, often living in rural areas within developing countries, herbal medicines, traditional treatments, and traditional practitioners are the main—and sometimes the only—source of health care. The affordability of most traditional medicines makes them all the more attractive at a time of soaring health care costs and widespread austerity.

In wealthy countries, TM meets an additional set of needs. People increasingly seek natural products and want to have more control over their health. They turn to TM to relieve common symptoms, improve their quality of life, and protect against illness and diseases in a holistic, nonspecialized way.

Incidentally, nearly a quarter of all modern medicines are derived from natural products, many of which were first used in a traditional medicine context. TM is thus a resource for primary health care, but also for innovation and discovery.

However, TM needs rigorous, scientific data to demonstrate its efficacy. It also needs evidence-based standards for quality and safety evaluation to support its appropriate regulation. I am happy to see included in this special feature of *Science* magazine, a series of perspectives on TM from a global team of experts, and would like to encourage more views to be shared and more robust research to be conducted in the area of TM in the future.

The general situation concerning the global use of TM was recently disseminated through the WHO Traditional Medicine Strategy 2014–2023. It makes clear that, to move into mainstream medicine on an equally trusted footing, TM needs a stronger evidence base. The need for stronger regulatory control covers not only the products, but also extends across the practice and practitioners. Updating and enhancing the strategy has allowed WHO to acquire a better understanding of how to boost the global integration of TM into health systems, to benefit individuals seeking the right care, from the right practitioner, at the right time.

The two systems of traditional and Western medicine need not clash. Within the context of primary health care, they can blend together in beneficial harmony, taking advantage of the best features of each system and compensating for certain weaknesses in each as well. In an ideal world, TM would be an option, a choice, offered by a well-functioning, people-centred health system that balances curative services with preventive care. The challenge is to give TM its appropriate place in an integrated health system, to help all practitioners understand its unique and valuable contribution, and to educate consumers about what it can and cannot do. In other words, we need to modernize this rich resource and cultural heritage, and put it in its proper place in today's world.

Margaret Chan, M.D.
Director-General, World Health Organization

Produced by the *Science*/AAAS Custom Publishing Office

A middle way for traditional medicine

Traditional medicine researchers are applying modern 'omics and the latest technologies in an attempt to standardize traditional treatments.

In discussions surrounding traditional healing techniques, a common perception is that those in the West most often take a reductionist approach to medicine, breaking down the body into ever-smaller parts in order to understand its inner workings. In the East, by contrast, medical practitioners are seen to take a more holistic view, regarding the body as a complex, integrated system and treating it as such.

At some point in the past, these two philosophies were certainly at odds. However, this seems less so to be the case today. The line between Eastern and Western medicine is blurring as "alternative" healing practices such as acupuncture, meditation, and yoga have become popularized in the West, and as evidence-based science finds a foothold in the East, particularly in the realm of drug discovery and development.

The rise of systems biology as a discipline, starting around five decades ago but gaining sharply in acceptance and popularity in the last 20 years, has created a slow but unambiguous shift in the Western research paradigm. Reductionism, although still a respected philosophy, is no longer consistently the preeminent methodology of choice in biological research. Researchers around the world are coming around to the notion that, while we can learn much from understanding the finest details at a molecular level, particularly when it comes to treating disease, a deeper knowledge of the interactions between systems and networks is essential.

Conversely, taking a purely holistic approach can produce its own challenges. This is particularly true when quality control of medicinal products and reproducibility of results comes into question. No matter the weight of historical, anecdotal data, drug regulatory agencies such as the U.S. Food and Drug Administration (FDA) will not allow new therapeutics for human treatment without verifiable scientific evidence. Although there are many challenges inherent in meeting this requirement, traditional medicine researchers are applying modern 'omics and the latest technologies in an attempt to standardize traditional treatments, especially through identification and isolation of bioactive compounds and careful analysis of their levels and activities in various herbal remedies.

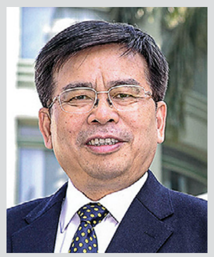
In Buddhism, the Middle Way is described as the route to enlightenment—a path found by balancing opposing views, accepting neither extreme, but rather investigating both sides and finding a middle ground. Perhaps a Middle Way can be found for traditional medicine, one that takes the best of East and West and brings them together for the benefit of all.

Alan Leshner, Ph.D.
CEO, AAAS
Executive Publisher, *Science*

Integrating traditional medicine into modern health care

Tai-Ping Fan, Ph.D.
(Guest project editor)
University of Cambridge, UK

Josephine Briggs, M.D.
National Center for Complementary & Alternative Medicine, NIH, USA



Liang Liu, M.D., Ph.D.
Macau University of Science & Technology, Macau SAR, China

Aiping Lu, M.D., Ph.D.
Hong Kong Baptist University, Hong Kong SAR, China

Jan van der Greef, Ph.D.
University of Leiden and TNO, The Netherlands

Anlong Xu, Ph.D.
Beijing University of Chinese Medicine, China

Almost every culture has its distinct herbal traditions, each with its indigenous plants and unique practices. But one premise unites them all—herbs have remarkable properties that make them a source of potentially powerful medicines.

Thanks to early explorers like Marco Polo (1254–1324), *materia medica* has been travelling between East and West for centuries. It is now important for us to harness the traditional medicines from across the globe. In Britain, the rich history of traditional medicine use was given credence in the early 1500s by the Herbalists Charter of Henry the VIII (1491–1547). His contemporary in China, Li Shizhen (1518–1593) was a great naturalist who spearheaded a 40-year research project that led to the publication of *Bencao Gang Mu*, a pharmacopoeia and also a treatise on botany, zoology, mineralogy, and metallurgy.

To make the case that traditional medicine has valuable insights for modern society, an independent editorial team was gathered consisting of experts in a range of topics related to traditional medicine research. This team compiled a unique collection of state-of-the-art perspectives from global experts on traditional medicine research, the first installment of which is presented in this special feature. Further exciting articles will be published early in 2015.

We have chosen traditional Chinese medicine (TCM) to illustrate the art and science behind the ancient practice of holistic healing, and how the good practices of quality control, pharmacology and toxicology testing, carefully designed clinical studies, and proper regulation are applicable to all traditional medicines.

This first issue introduces the WHO Traditional Medicine Strategy (2014–2023), highlighting the global scientific challenges and showing how a systems biology approach can be applied to diagnosis, leading to integrated network-based medicine. Recent advances in mechanistic studies of acupuncture are also discussed. Some of the exciting areas in TCM research include the therapeutic potential of herbal remedies against influenza, cancer, diabetes, and cardiovascular diseases; the exploration of gut microbiota-targeted dietary interventions against chronic inflammation; and the study of the biological activities of complex polysaccharides present in medicinal plants. Chemogenomics and network pharmacology have been applied to predict molecular targets and decipher the mechanisms of action of pure compounds or phytocomplexes found in combinatorial herbal formulas. A better understanding of the philosophy of synergetic interactions of *Jun, Chen, Zuo,* and *Shi* classes of Chinese *materia medica* used in traditional formulations has led to a simplified *Jun-Shi* compatibility drug discovery strategy model.

Evaluating the safety of herbal medicines is critical to their wider acceptance as valid therapeutic agents. Integrated toxicological approaches have been successfully applied in this area, for instance to identify antifibrotic and profibrotic substances in certain medicinal plants. As research into the broader application of traditional medicine continues, newer 'omics technologies and poly-pharmacokinetics will also play an increasing role in bridging the gap between the personalized approach of Chinese medicine theory and modern clinical research methodology.

Acknowledgments
We are particularly grateful to Zhu Chen, vice-chairman of the Standing Committee of the National People's Congress of the People's Republic of China for inspiring us to undertake this project, to WHO Director-General Margaret Chan and her team, Commissioner Guoqiang Wang of the State Administration of Traditional Chinese Medicine, and AAAS CEO Alan Leshner for their vision and support for this special feature. Thanks are also due to all authors, referees, advisors, and sponsors for charting the journey ahead to translate ancient traditional medicines into the therapies of tomorrow.

Produced by the *Science*/AAAS Custom Publishing Office

The WHO Traditional Medicine Strategy 2014–2023: A perspective

Authors:
Zhang Qi[1*] and
Edward Kelley[2]

There has been a continuing demand for, and popular use of, traditional and complementary medicine (T&CM) worldwide. In some developing countries, native healers remain the sole or main health providers for millions of people living in rural areas. For instance, the ratio of traditional health practitioners to citizens in Africa is 1:500, whereas the ratio of medical doctors to citizens is 1:40,000 (*1*). In the Lao People's Democratic Republic, 80% of the population live in rural areas, with each village being serviced by one or two traditional health practitioners (*2*). Over 100 million Europeans are currently users of T&CM, with one-fifth being regular users; a similar proportion choose health care that includes T&CM (*3*). According to a national survey in China, practitioners of traditional Chinese medicine received 907 million visits from patients in 2009, which accounts for 18% of all medical visits to surveyed institutions. Further, the number of traditional Chinese medicine inpatients was 13.6 million, or 16% of the total in all hospitals surveyed (*4*).

In a few countries, certain types of traditional medicine (TM) have been completely integrated into the health care system, including China, the Democratic People's Republic of Korea (North Korea), the Republic of Korea (South Korea), India, and Vietnam. In China, for instance, traditional Chinese medicine and conventional medicine are practiced alongside each other at every level of the health care service, and public and private insurance cover both forms of treatment (Box 1).

In many other countries, T&CM is partially integrated into the national health system, while in some countries there is no integration at all.

Recent changes, emerging challenges, and needs

Much has changed since the last World Health Organization (WHO) global strategy document was released in 2002. More and more countries are coming to accept the contribution that T&CM can make to the health and well-being of individuals and the comprehensiveness of their health care systems. In the period 1999 to 2012, the number of member states of WHO with national policies covering TM has increased significantly. This includes countries better regulating herbal medicines or creating national research institutes to study T&CM (*5*).

Governments and consumers are becoming more open to broader aspects of T&CM practices and to considering them as an integrated part of health service delivery. In Africa, the number of national regulatory frameworks increased from one in 1999/2000 to 28 in 2010 (*6*). Across the Atlantic, the Ministry of Health in Brazil has developed a national policy on integrative and complementary practices (*7*), while in the eastern Mediterranean region, five member states report having regulations specifically for T&CM practitioners (*5*). Member states in the southeast Asia region are now pursuing a harmonized approach to education, practice, research, documentation, and regulation of TM (*5*); in Japan, 84% of Japanese physicians use Kampō (Japanese traditional medicine) in daily practice (*8*). In Switzerland, certain complementary therapies have been reinstated into the basic health insurance scheme available to all Swiss citizens (*9*).

Despite significant advances, the regulation of T&CM products, practices, and practitioners is not occurring at an equal pace (*5*). Member states report that faster progress is being made in the regulation of herbal medicines, while that for T&CM practices and practitioners is lagging. Of concern is that the safety, quality, and efficacy of T&CM services cannot be assured if there is not appropriate regulation of practices and practitioners. This situation presents a serious challenge for many member states, where a lack of knowledge and experience exists regarding the formulation of national policy, leading to weak or absent regulation and a lack of proper integration of T&CM services into the health service delivery system. It also reflects the need of all member states to push WHO to update its global strategy on TM.

The WHO Traditional Medicine Strategy: 2014–2023

Responding to the needs and challenges identified by member states and building on the work done under the *WHO Traditional Medicine Strategy 2002–2005* (*10*), the updated strategy for the period 2014–2023 devotes more attention than its predecessor to health services and systems, including T&CM products, practices, and practitioners. The key objectives of the updated strategy are summarized below.

Objective 1: *To build the knowledge base for active management of T&CM through appropriate national policies.* There is a great diversity of products, practices, and practitioners in T&CM. The first strategic step towards achieving this objective is to understand and recognize the role and potential of T&CM. The strategy recommends that member states acknowledge and appraise, in detail, which types of T&CM are used by their populations and devise their own country profile for T&CM practices. As the marketplace for T&CM becomes more global, harmonization and cooperation will become more important.

The second strategic step under this objective recommends that member states strengthen knowledge generation, collaboration, and sustainable use of T&CM resources, including intellectual and natural resources.

Objective 2: *To strengthen quality assurance, safety, proper use, and effectiveness of T&CM by regulating T&CM products, practices, and practitioners.* The first strategic element under this objective is to recognize the role and

Materials that appear in this section were not reviewed or assessed by *Science* Editorial staff, but have been evaluated by an international editorial team consisting of experts in traditional medicine research.

[1]Coordinator, Traditional and Complementary Medicine Unit, Service Delivery and Safety Department, World Health Organization, Geneva, Switzerland
[2]Director, Service Delivery and Safety Department, World Health Organization, Geneva, Switzerland
[*]Corresponding Author: zhangqi@who.int

importance of product regulation. The emphasis should be on the monitoring and implementation of established regulations of TM products. Since herbal medicines are now used internationally, products often used in parts of the world other than that in which they were originally grown, developed, or manufactured. This highlights the importance of considering different legislative frameworks in different countries, and ensures that information on quality and safety is shared so that products are used appropriately.

The second strategic direction is to recognize and develop T&CM practice and practitioner regulations for education and training, skills development, services, and therapies. As more countries develop policies and regulatory frameworks, there is a need to evaluate their effectiveness and identify ways in which challenges regarding practice and practitioner regulations can be addressed by benchmarking against appropriate reference standards.

BOX 1. Traditional medicine health service integration in China.

In China, there are about 440,700 health care institutions providing TM services, with 520,600 patient beds, including all levels of TM hospitals and general hospitals, clinics, and health stations in urban and rural areas. About 90% of general hospitals include a TM department and provide TM services for all patients. TM medical institutions are governed by the same national legislation on medical institutions as conventional medical institutions. TM practitioners are allowed to practice in both public and private clinics and hospitals. The public is free to choose their preferred form of health care services, or follow the advice of their doctors (*12*).

Objective 3: *To promote universal health coverage by integrating T&CM services into health care service delivery and self-health care*. One of the most significant questions raised about T&CM in recent years is how it might contribute to universal health coverage by improving service delivery in the health system, particularly primary health care. A first step is to capitalize on the potential contribution of T&CM to improve health services and health outcomes. Mindful of the traditions and customs of peoples and communities, member states should consider how T&CM might support disease prevention or treatment as well as health maintenance and health promotion. This process should be consistent with safety, quality, and effectiveness standards and in line with patient choice and expectations. Based on each country's realities, it is recommended that models for integrating T&CM into national health systems should be explored.

Next, it is important to ensure that consumers of T&CM can make informed choices about self-health care. In many member states, self-selection of T&CM products accounts for a large part of the T&CM market. Education of consumers, together with ethical and legal considerations, should support and shape the key aspects of informed choice for T&CM intervention.

The WHO resolution WHA67.18 urges member states to adapt, adopt, and implement the *WHO Traditional Medicine Strategy 2014–2023* as a basis for national T&CM programs or work plans and to report to WHO on progress in implementing the strategy. The resolution also encourages WHO to support member states in the implementation of the strategy in the coming decade (*11*).

Conclusions

Around the world, T&CM continues to grow in popularity. Progress in the regulation of T&CM is gaining momentum, even as that of T&CM practices and practitioners advances at a somewhat slower pace. Safety, quality, and effectiveness of T&CM services is paramount, but cannot be ensured if appropriate regulation of practices and practitioners is not in place. The goals of *WHO Traditional Medicine Strategy 2014–2023* are to support member states in harnessing the potential contributions of T&CM to health, wellness, people-centred health care, and universal health coverage, while also promoting safe and effective use of T&CM through the regulation, research, and integration of T&CM products, practices, and practitioners into the health system, as appropriate.

It should be emphasized that given the great diversity of products, practices, and practitioners in T&CM among the member states, it is important to enhance international communication and collaboration in sharing knowledge and practices, in developing and exchanging scientific knowledge and training programs, and in sharing experiences in developing and implementing policies and regulations. Also, as the marketplace for T&CM becomes more globalized, the quality, safety, proper use, and efficacy of T&CM in different nations need to be harmonized and standardized utilizing evidence-based science.

References

1. A. A. Abdullahi, *African J. Trad., Comp. and Altern. Med.* **8**, 115 (2011).
2. Lao Ministry of Health and World Health Organization. *Health Service Delivery Profile, Lao PDR, 2012* (Compiled in collaboration between WHO and Ministry of Health, Lao PDR, 2012), http://www.wpro.who.int/health_services/service_delivery_profile_laop dr.pdf.
3. European Information Centre for Complementary & Alternative Medicine (http://www.eiccam.eu/home.php?il=1&l=eng).
4. State Administration of Traditional Chinese Medicine, *Report of a survey on T&CM basic situation in 2009* (2011).
5. WHO Traditional Medicine Strategy 2014-2023 (World Health Organization, Geneva, 2013), pp. 15-56.
6. World Health Organization, *Progress report on decade of traditional medicine in the Africa region* (WHO African Region (AFRO), Brazzaville, 2011).
7. Retrieved from http://bvsms.saude.gov.br/bvs/publicacoes/pnpic_access_expansion_initiative.pdf.
8. E.C. Moschik *et al.*, Usage and Attitudes of Physicians in Japan Concerning Traditional Japanese Medicine (Kampo Medicine): A Descriptive Evaluation of a Representative Questionnaire-Based Survey. *Evidence-Based Complementary and Alternative Medicine*. **2012**, (Article ID 139818, 13 pgs, 2012).
9. Swiss Confederation, *Five CAM methods eligible for reimbursement under specific conditions for a provisional period of six years*. (2011; www.bag.admin.ch/aktuell/00718/01220/index html?lang=de&msg-id=37173).
10. *WHO Traditional Medicine Strategy 2002–2005* (World Health Organization, Geneva, 2002).
11. World Health Organization, *WHO Governing Body Documentation Official Records*, http://apps.who.int/gb/or/.
12. Government of China, National Bureau of Statistics of China. *China Statistical Yearbook 2011: Chinese Medicine (1987–2010)* (The State Administration of Traditional Chinese Medicine, China, 2011).

A global scientific challenge: Learning the right lessons from ancient healing practices

Author:
Josephine P. Briggs

Health is a fundamental human value. Consequently, most cultures have sought after and used a broad range of healing practices. In both developed and developing economies, the practices of modern medicine exist side-by-side with traditional approaches and alternative remedies. For many living in developing economies, traditional healers and herbal remedies are the only source of available health care. In contrast, developed economies typically use these approaches as an optional complement to modern medicine, driven by patient preference. However, in both China and India, the ancient medical traditions—traditional Chinese medicine and Ayurvedic medicine—have flourished either in parallel or integrated with advanced modern care. Currently, in North America and Europe certain ancient healing practices—such as acupuncture, traditional Chinese medicine, massage, and meditation—have generated increasing interest and are seen as gentler, "low-tech" complements to conventional care.

The persistence of such traditional practices in these settings suggests we have much to learn from them. Modern scientific methods can offer means to examine traditional practices. In this brief perspective, a few examples of traditional remedies are discussed to illustrate the issues we face in thinking about the intersection between modern medicine and traditional healing practices.

Malaria and botanical-based therapies

About half of the pharmaceuticals developed over the last two decades and approved by the U.S. Food and Drug Administration (FDA) were either natural products, synthetic derivatives, or had at their core a prototype molecule derived from a natural product (*1*). Continuing to development therapeutics from natural sources is imperative for finding new treatments, and healing traditions can provide insight into such resources. Moreover, many modern therapeutics exploit the ability of plants to synthesize a wide range of small molecules of great variety and complexity. Plant-based medicines remain important sources of therapeutics for much of the world's population, and plant-derived products remain a major source of new therapeutic small molecules.

Treatment of malaria is a case in point. Modern malaria treatment started with the discovery of *Cinchona* (Rubiaceae) bark, which was reportedly being used in South American native traditional healing practices and was brought back to Europe by Jesuit missionaries in the 17th century. Until the mid-1990s, virtually all treatments for malaria were molecular entities based on the terpenoid structure of the *Cinchona* alkaloids. This includes quinine, chloroquine, and the synthetic derivative mefloquine. The alkaloids are abundant in *Cinchona* bark, up to 15 percent by weight, and quinine itself can represent half or more of the alkaloid content (*2*). Simple tea extracts of *Cinchona* bark have substantial antimalarial activity. Quinine-based compounds remain critical for both the treatment of malaria and for prophylaxis, but the emergence of resistance, particularly for *Plasmodium falciparum*, has created enormous urgency to develop new agents.

The next chapter in the development of antimalarial therapy belongs to *Artemisia annua*—a vigorous weedy annual which is widespread throughout the world (Figure 1A). *Artemisia annua* is known in Chinese herbal tradition as *qinghao* and as sweet wormwood in Europe. The development of artemisinin-based antimalarials represents one of the great recent victories for ethnomedicine. The discovery was a complex team effort, initially led by the Chinese, later bringing in Western nonprofit and governmental entities and pharmaceutical companies (*3, 4*). The story illustrates how healing traditions can point scientists in a direction to find new medicines, but also exemplifies how challenging developing therapeutics from plants can be.

The Chinese government funded a major screening effort, Project 523, which was undertaken to test a large number of herbs, using a mouse model of malaria. Using a labor intensive methodology, they studied thousands of herbs, hundreds of extraction "recipes," and had hundreds of hits. Moving from these hits to the initial isolation and characterization of a bioactive compound required enormous persistence. The effort to find a new treatment could easily have failed; the reports suggested that drug responses were fleeting at times and difficult to reproduce. However, a gentle extraction method that maintained activity of the active principle was finally developed by Tu YouYou (*3*). In contrast with the *Cinchona* alkaloids, artemisinin (Figure 1B) is present in only low concentrations, sometimes as low as 0.05% by weight (*2*). Additionally, it is detected only in *Artemisia annua*, but not in other *Artemisia* species. Its unusual endoperoxide structure is critical for activity, but is relatively unstable under many extraction conditions. Nevertheless, the effort succeeded, yielding a small molecule that is now the cornerstone of malaria treatment.

Acupuncture and chronic pain

Acupuncture is an integral part of Asian medical traditions, where it is widely used in traditional practice settings and its efficacy in relieving pain is taken as a given; however, acupuncture's introduction into American and European health care settings, while welcomed by many patients and providers, has encountered substantial skepticism from medical professionals. A sizable number of clinical trials have been implemented to explore its value, many of which are focused on pain management. These have included both

Director, National Center for Complementary and Alternative Medicine
National Institutes of Health, Bethesda, MD
briggsj@mail.nih.gov

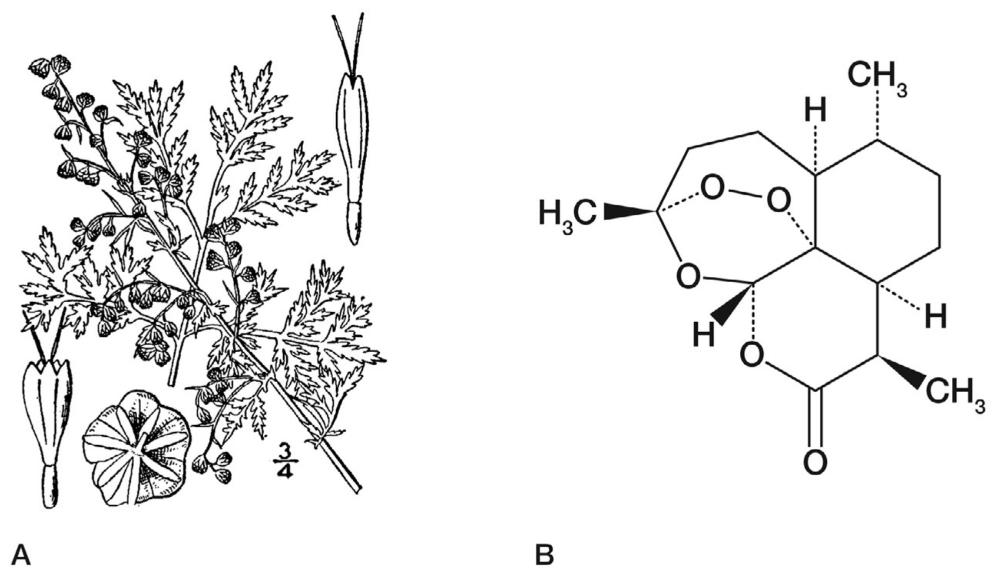


FIGURE 1. Artemisinin. (A) *Artemisia annua*, also known as sweet wormwood or *qinghuo*. **(B)** The chemical structure of artemisinin.

efficacy and effectiveness trials. Efficacy trials generally compare acupuncture to a sham control designed to be indistinguishable to the subject from traditional acupuncture. The typical sham controls use the same ritual, the same practitioner reassurance, and the same counter-irritant effect of needles or pressure points as the comparison group (although the needles may be replaced with non-penetrating devices and the insertion points chosen do not lie on treatment meridians). Effectiveness trials typically compare a subject's response to different treatments, which are randomly assigned and include either acupuncture or standard care.

A recent, systematic review of 29 trials, with approximately 18,000 study participants, resulted in some clear conclusions: The magnitude of acupuncture's effect depends upon which group is used for a comparison (*5*). Specifically, when acupuncture was compared to no acupuncture (in effectiveness studies), the benefit appears to be quite sizable, approximately 50% reduction in pain severity. In contrast, when acupuncture is compared to a sham treatment (in efficacy studies), more modest effects are observed (Figure 2). Although statistical significance is achieved, the reduction in pain severity is not as substantial, typically only 20%. Based on this analysis, it seems reasonable to conclude that needling itself may be contributing to acupuncture's pain-reducing effects, and that the overall benefit is heavily dependent on context—on the reassurance and expectation produced by the acupuncture ritual.

What does this mean for clinical practice? Here, the arguments erupt. Is a contextual effect (some would call it a placebo) that relieves pain and reduces the need for medication an acceptable form of treatment? This is still, for many Western practitioners, a quandary to which there is no simple answer. Building a better biological understanding based on the neuroscience of pain may provide some common ground. As we learn more about the central pain circuits, the mechanisms underlying acupuncture's effects—specific and nonspecific—can also be teased out. Acupuncture appears to modulate the central circuitry for pain (*6*), partially from the peripheral actions of adenosine (*7*) and partially from the centralized actions of endorphins (*8*). Moreover, there may be overlap in the descending pain circuits recruited by the specific and contextual effects.

Ultimately the goal of the research is clear. We need better treatments for pain without the sedative, narcotic, and addictive effects of the available drugs. Understanding this ancient tradition is a good place to turn for insights that will improve pain treatment.

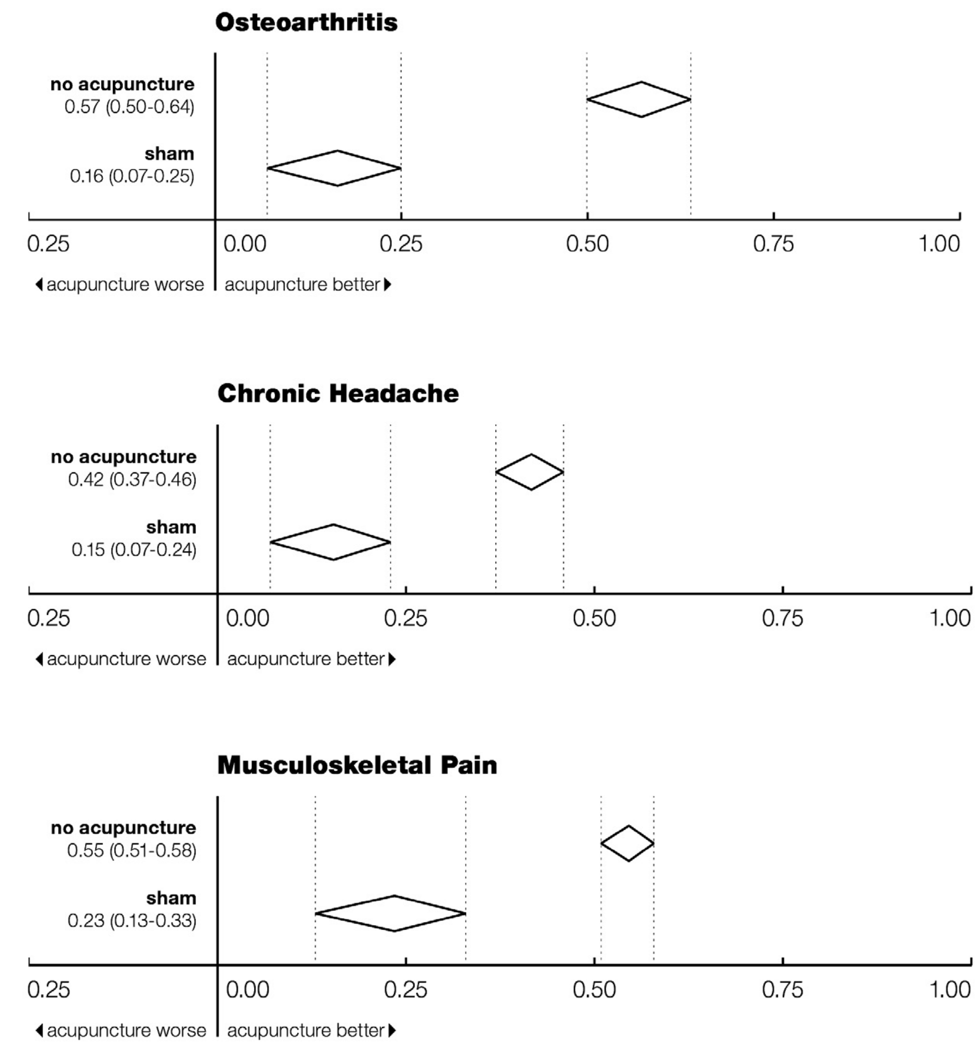
Traditional versus modern medical systems

A major difference between resource intensive and resource poor environments is the extent to which common complaints of daily living are viewed as medical problems requiring intervention, help, and treatment. Medicalization has been defined as, "the process by which human conditions and problems come to be defined and treated as medical conditions, and thus become the subject of medical study, diagnosis, or treatment" (*9*). As health care providers in resource-intensive environments, we often take for granted the medicalization of a wide range of complaints—sadness, worry, fatigue, musculoskeletal discomforts, and even restless legs—as necessary and normal.

Comparisons of traditional and modern health systems challenge these assumptions. Modern medicine has created a set of symptoms-based diagnostic categories for a range of common problems that differ greatly from those in traditional health systems. Diagnostic criteria, based on symptoms and limited physical findings but lacking laboratory diagnostic criteria, have been developed for a variety of physical complaints, such as chronic fatigue syndrome and chronic functional pain syndromes such as fibromyalgia, interstitial cystitis, vulvodynia, and chronic prostatitis. These diagnostic categories carry clinical insights about symptoms that cluster together, and sometimes patients can benefit. The

Produced by the *Science*/AAAS Custom Publishing Office

FIGURE 2. Acupuncture effects compared with controls. The results of a meta-analysis of 29 high-quality randomized clinical trials of acupuncture are shown for three conditions. Differences in the average standardized mean (with 95% confidence intervals) for treatment relative to control is shown. When compared to no treatment, acupuncture produces striking improvement; however, when compared to sham treatments, the effect is more modest (*5*).



diagnoses may provide some clarity and community support. Nevertheless, although there is some evidence for efficacy of pharmaceutical treatments for some such conditions, often these disorders respond inconsistently or poorly to available treatments. There is also clinical concern that sometimes these diagnoses can contribute to an expectation of chronic functional impairment.

Traditional diagnoses often emphasize a temporary imbalance and promote an expectation that the subject will return to health. Although many patients with these conditions seek alternative remedies, most of the evidence of benefit is anecdotal. In resource-poor environments, people almost certainly suffer from the same set of symptoms, and anecdotally, at least, these complaints may sometimes be effectively addressed through the care of traditional healers. We are currently supporting a small number of trials that address whether the emotional and social support of interventions such as tai chi (*10*), yoga, or mindfulness-based meditation may capture some of the benefit of the healing traditions. Clearly, Western medicine does not have all the answers, and systems of care that allow thoughtful integration of healing traditions with modern medicine may offer help to troubled patients.

References

1. D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **70**, 461 (2007).
2. P. M. Dewick, *Medicinal Natural Products: A Biosynthetic Approach*. (Wiley, New York, ed. 3, 2009). pp. 335–336.
3. Y. Tu, *Nat. Med.* **17**, 1217 (2011).
4. D. G. Dalyrmple. *Artemisia annua, Artemisinin, ACTs and Malaria Control in Africa: Tradition, Science and Public Policy*. (Politics and Prose Bookstore, Washington, DC, 2012).
5. A. J. Vickers *et al*., *Arch. Int. Med.* **172**, 1444 (2012).
6. J. Kong *et al*., *Neuroimage* **47**, 1066 (2009).
7. T. Takano *et al*., *J. Pain* **13**, 1215 (2012).
8. J. S. Han, *Neurosci. Lett.* **361**, 258 (2004).
9. Medicalization entry. Wikipedia. Accessed at: http://en.wikipedia.org/wiki/Medicalization on June 21, 2014.
10. C. Wang *et al*., *NEJM* **363**, 743 (2010).

Produced by the *Science*/AAAS Custom Publishing Office

East is East and West is West, and never the twain shall meet?

Authors: Yan Schroën[1,3], Herman A. van Wietmarschen[1,2,], Mei Wang[1,5], Eduard P. van Wijk[1,4], Thomas Hankemeier[4], Guowang Xu[1,6], Jan van der Greef[1,2,4,5*]

Western medicine and Chinese medicine developed within the context of different cultures and perspectives of the natural world. The more reductionistic approach of Western biomedical sciences has generated tremendous knowledge of anatomy, physiology, histology, genetics, and biochemistry, while the phenomenological approach of Chinese medicine has produced a more holistic understanding of biology. The two concepts are complementary, and combining them to optimally balance detail and context could generate a highly rewarding step forward for medicine.

A diversity of perspectives on life and consciousness has developed across humanity's different cultures. In the Western Hemisphere, a key development was the affirmation by Greek Ionian scholars such as Thales, Pythagoras, and Archimedes that it was not gods, but rather laws of nature, that create and organize our reality. The modern concept of the laws of nature emerged in the seventeenth century through the work of scholars such as Keppler and Galileo, with the most notable contributions coming from Newton and from Descartes, who emphasized a duality between the mind and the physical body.

Philosophers have pondered whether more than one set of laws was possible. It seems that our conception of natural laws may depend on our approach to understanding reality. Hawking and Mlodinov introduced the notion of model-dependent realism, which posits that a physical theory or worldview is a model with a set of rules that connects the elements of the model to observations (*1*). That is, in the words of Hawking and Mlodinov, "There is no picture- or theory-independent concept of reality," and every model is only an approximation of reality.

The modern Western scientific model arose in the context of historical and cultural developments that enabled philosophical pursuits and provided fertile ground for philosophers of science. A different approach to understanding reality and the laws of nature arose in Eastern cultures, such as China. Both models can be considered valid, each with its own model-dependent realism.

Bridging the gap

Although there are many similarities between the Greek and Chinese concepts of health and medicine, the medical systems that arose in the West and in the East are quite distinct. Most notably, a highly reductionist, detailed view dominates in the West, whereas a more phenomenological, descriptive, and systems-based view holds sway in China. In recent decades, Western systems thinkers have started to combine theories from a variety of disciplines, developing an expanded systems view of medicine. Systems thinking, and in particular systems biology, have been recognized as the scientific bridge between Western medicine and traditional medicine models, including traditional Chinese medicine (TCM) (*2, 3*).

Figure 1 illustrates how systems-based theories can bridge Eastern and Western models, as well as connecting ancient and modern ideas. The left forward image shows a dynamic correlation network of interactions between various genes, proteins, and metabolites. This nodal network reflects the particularized understanding of the complexity of biochemical pathways and the dynamic organization of the body that characterize Western biomedical science. The right forward imagery is a drawing of the Taoist Inner Landscape. In keeping with ancient Taoist tradition, the drawing provides a poetic description of the complex relationships among the various organ functions of the body. The background of the figure merges two very well-known, almost archetypical, symbols of systems thinking: the Vitruvian Man (*Le proporzioni del corpo umano secondo Vitruvio*) and the *Taiji* (太极, the literal translation of which is "great pole"). The Vitruvian Man is by Leonardo da Vinci, a visionary and pioneer of the evidence-based scientific view of the universe. A man is pictured within a square, which reflects the terrestrial aspect of humanity, and a circle, which represents the spiritual realm. The *Taiji* (often called the Yin-Yang symbol in the West) represents the Eastern, Taoist tradition of systems thinking. It depicts a dynamic relationship between the two components of a duality that encompasses the known universe. Interestingly, the *Taiji*, which symbolizes humanity as part of an eternal universe, has all the properties of a fractal.

Amalgamation in action

Figure 2 depicts an amalgamation of Western and Eastern medical systems, a process that we call systems medicine. The left side of the figure shows a simplified, hierarchical view of molecules being organized into cells, with further consolidation into tissues, organs, and, ultimately, a whole organism. This illustrates the bottom-up approach practiced in Western biomedical sciences. It has produced a tremendous amount of knowledge of anatomy, physiology, cells, genes, and biochemistry. It has also created physicians with highly specialized, albeit arguably fragmented, knowledge. In the Western scientific model, data are collected to generate information, knowledge, and, ultimately, a form of wisdom. By contrast, traditional medical systems, most prominently TCM, have focused on gaining a holistic understanding of systems, and on applying that wisdom in a top-down manner in the search for knowledge, information, and data that may increase the understanding of the web of life. Somewhere in the middle these two worldviews meet and this nexus has the potential to yield a valuable combination in which detail and context are optimally balanced.

[1]Sino-Dutch Centre for Preventive and Personalized Medicine, P. O. Box 360, 3700 AJ, Zeist, The Netherlands
[2]TNO, P.O. Box 360, 3700 AJ, Zeist, The Netherlands
[3]Oxrider, Education and Research, Diessenseweg 51, Hilvarenbeek, The Netherlands
[4]Division of Analytical Biosciences, LACDR, Leiden University, P.O. Box 9502, 2300 RA Leiden, The Netherlands
[5]SU BioMedicine, Utrechtseweg, Zeist, the Netherlands
[6]Dalian Institute of Chemical Physics, Chinese Academy of Sciences, Dalian, China
*Corresponding Author: jan.vandergreef@tno.nl

Materials that appear in this section were not reviewed or assessed by *Science* Editorial staff, but have been evaluated by an international editorial team consisting of experts in traditional medicine research.

FIGURE 1. An artist impression of the ancient and modern bridge between Chinese and Western medicine.

One way to bridge the two worldviews is through unification of diagnosis, based on an integration of the collections and arrangements of symptoms and signs. Western biomedical advances offer a plethora of biomarkers that can be detected and measured with advanced equipment, while Chinese medicine contributes knowledge about the dynamic relationships among signs and symptoms. The right side of Figure 2 provides an example of this inter-relationship for rheumatoid arthritis (RA). In Chinese culture, RA is classified as a "*Bi Zheng*," a so-called painful obstruction syndrome. In TCM diagnosis every condition is primarily distinguished according to eight basic principles: External-Internal, Heat-Cold, Excess-Deficiency, and Yin-Yang. Figure 2 focuses on the Cold-Heat differentiation.

The signs and symptoms of RA are universally represented across peoples independent of culture, although variations in concepts and emphasis can be seen. In TCM, RA patients can be subdivided based on the predominance of "hot" versus "cold" symptoms. Examples of "hot" symptoms, as illustrated in Figure 2, are thirst, fever, irritability, restlessness, warm feeling, dry mouth, and pain that is relieved by cold, while "cold" symptoms include clear urine, sharp pain, stiff joints, and pain that is relieved by warmth. This systemic approach may help biomedical researchers to distinguish biological subtypes of RA in a manner that could lead to personalization of medical care; firstly, through more personalized lifestyle advice, and in the long term, through the application of modern biomedical technology in studies of RA subtypes. Ultimately, recognizing the particular individualized presentation of RA across different patients based on a systemic approach may improve treatment choices and outcomes.

Recently, research teams have begun the process of integrating Western and Eastern notions of medicine for RA. For example, Van Wietmarschen and colleagues (*4*) used a questionnaire to differentiate distinct "cold" and "hot" RA subtypes. These two patient groups display differences in the regulation of apoptosis, in CD4+ T cell gene expression levels, and in plasma and urine metabolite profiles. In another study, 11 acylcarnitine metabolite variants associated with differences in muscle breakdown was used to distinguish between the "cold" and "hot" RA subtypes (*5*).

In similar recent work on pre-diabetes, Wei and colleagues (*6*) examined blood and urine samples from patients categorized by Chinese subtypes of pre-diabetes, namely *qi* and yin deficiency with or without dampness, and *qi* and yin deficiency with stagnation. Numerous sugar and amino acid level differences were recorded, indicating that the subtypes are characterized by variation in carbohydrate metabolism and renal function.

Several other studies have also shown that biological mechanisms can be correlated with TCM-based groupings in patients diagnosed in Western medical systems (*7–10*).

The path ahead

In the above studies, TCM subtyping and Western diagnostic criteria coincided. This suggests that symptom pattern questionnaires could reliably standardize the segregation of patients into TCM subgroups (*11*). The comprehensive symptom questionnaire used in the RA study was based on the TCM perspective of arthritis as a bi-syndrome. Following completion of the questionnaire, the data was subjected to a principal component analysis, revealing one principal component related to the concepts of "internal" and "external," plus another related to "cold" and "hot" (*11*). These findings support the contention that TCM concepts have a basis in actual biological variation among patients. The current challenge is to uncover nonlinear relationships between the diagnostic symptom clusters revealed by the questionnaire results.

We believe that Western diagnostics would benefit greatly from the integration of broader knowledge of relationships between symptoms, including consideration of TCM descriptions of syndromes. TCM descriptions offer potential directions for detailed, explanatory biomedical research, bringing us closer to a biopsychosocial model of health in which more and more relationships between diseases, psychology, and behavior are uncovered (*12*). Arguably, the dearth of understanding of the dynamics of systems presents the greatest opportunity for improvement in Western health care diagnostics. It is a topic that will only grow in importance as the focus in health care shifts from the treatment of acute disease to the long-term management and prevention of chronic diseases. Among the more promising developments that may improve our understanding of system dynamics are the application of nonlinear dynamic modeling techniques to the study of coherent oscillations in the brain (*13*), examination of the synchronization of physiological rhythms such as heart beat and breathing rhythms (*14*), and the study of metabolic processes that show oscillatory behavior (*15*). Another

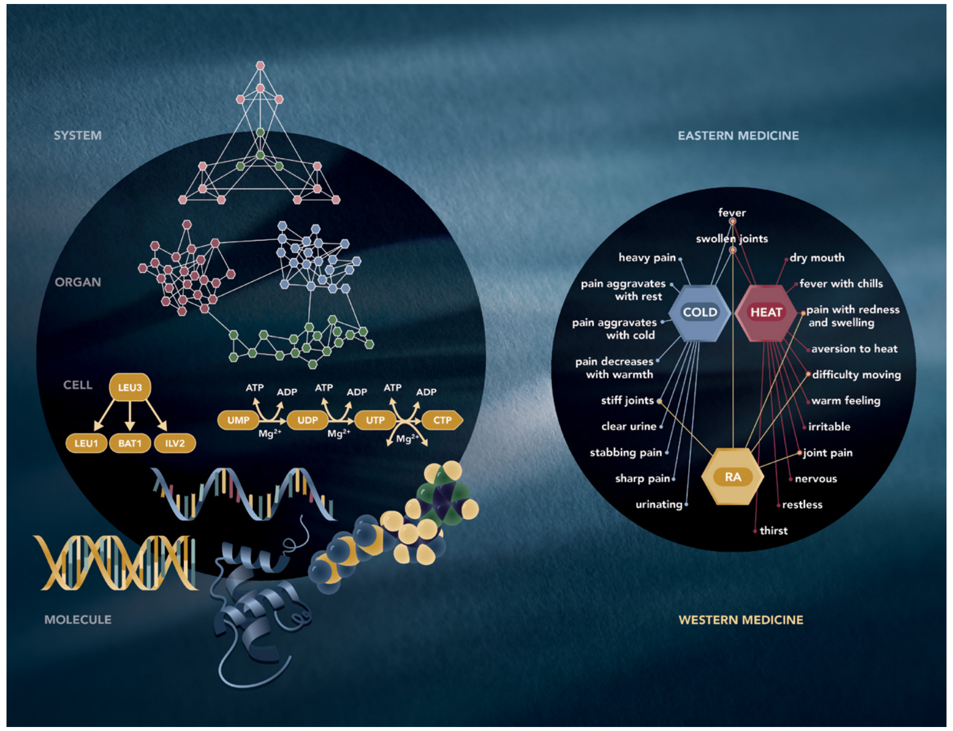


FIGURE 2. Systems medicine. A hierarchical systems view on human biology (left)–scientific studies in Western medicine develop typically via a biochemistry/pathway bottom-up approach, while in Chinese medicine, a top-down dynamic symptom relationship approach is more common. The right image illustrates the diagnostic bridge between symptom relations in Chinese Medicine bi-syndrome (top) and Western medicine (bottom) for rheumatoid arthritis.

intriguing area being examined is the coherent, spontaneous ultra-weak photon emission patterns of organisms (*16, 17*). Recent work suggests that photon distribution dynamics may provide insights into regulatory coherence at a high systems level (*18, 19*). Indeed, these coherent light functions may be directly involved in communication in addition to influencing biochemical networks (*20, 21*).

It should also be clear that modern quantitative technologies developed in the West have a great deal to offer to Chinese diagnostics. Especially relevant are methodologies that provide information about the large-scale organization of systems as well as the dynamics of such organization (Figure 2).

Integration of Western and Chinese medicine thinking has enormous potential for synthesizing modern technological and social innovation. Although Chinese and Western medicine are perceived as wholly distinct paradigms today, they are poised to merge in the arena of personalized systems medicine, wherein patients can take a greater role in managing their own health and wellness. Human-human relationships are critical for diagnosis and intervention in a biopsychosocial context, with health care providers supporting patients through an empathic coaching role. The integration of Western and Chinese medicine can be much more than the sum of the parts: it can accelerate the shift from disease management to health promotion that is presently taking place in health care systems around the world. Although, as Kipling states in the opening lines of his famous poem, "East is East and West is West" (*22*), at least in the realm of diagnostic medicine, these two world cultures have met.

References

1. S. Hawking, L. Mlodinov, *The Grand Design* (Bantam Press, New York, 2010).
2. M. Wang *et al.*, *J. Phytother. Res.* **19**, 173 (2005).
3. J. van der Greef, H. van Wietmarschen, Y. Schroën, M. Wang, T. Hankemeier, G. Xu., *Planta Med.* **76,** 1 (2010).
4. H. van Wietmarschen *et al.*, *J. Clin. Rheumatol.* **15**, 330 (2009).
5. H. van Wietmarschen et al., *PLOS ONE* 7:e44331 (2012).
6. H. Wei *et al.*, *Mol. Biosyst.* **8**, 1482 (2012).
7. S. Li *et al.*, *IET Syst. Biol.* **1**, 51 (2007).
8. C. Matsumoto, T. Kojima, K. Ogawa *et al.*, *Evid.-Based Compl. Altern. Med.* **5,** 463 (2008).
9. B. Patwardhan, G. Bodeker, *J. Altern. Compl. Med.* **14**, 571 (2008).
10. Q. Wang, S. Yao, *Am. J. Chin. Med.* **36**, 827 (2008).
11. H. van Wietmarschen *et al.*, *PLOS ONE* **6**:e24846 (2011).
12. G. Engel, *Science* **196**, 129 (1977).
13. G. Buzsáki, A. Draguhn, *Science* **304**, 1926 (2004).
14. L. Glass, *Nature* **410**, 277 (2011).
15. J. Bass, J. S. Takahashi, *Science* **330**, 1349 (2010).
16. F. A. Popp, L. Beloussov, *Integrative Biophysics: Biophotonics* (Kluwer Academic Publishers, Dordrecht 2003).
17. R. van Wijk, *Light in Shaping Life: Biophotons in Biology and Medicine* (Ten Brink, Meppel, 2014).
18. R.P. Bajpai, E. P. A. van Wijk, R. van Wijk, J.van der Greef, *J. Photochem. Photobiol. B.* **129** 6 (2013).
19. R. van Wijk, E. P. A. van Wijk, H. A. van Wietmarschen, J. van der Greef, *J. Photochem. Photobiol. B.* **S**1011 (2013).
20. G. Albrecht-Buehler, *Proc. Natl. Acad. Sci. USA* **102** 5050 (2005).
21. D. Fels, *PLOS ONE* **4**, e5086 (2009).
22. R. Kipling, *The Ballad of East and West* (Sterling Publishing Co. Inc., New York, 1889).

Acknowledgments
The authors thank Charlotte Lokin for producing the artwork shown in Figure 1.

Produced by the *Science*/AAAS Custom Publishing Office

Zheng: A systems biology approach to diagnosis and treatments

Authors:
Yonghua Wang and Anlong Xu*

Traditional Chinese medicine (TCM) is an ancient medical practice system which emphasizes regulating the integrity of the human body and its interrelationship with natural environments. As a key concept in TCM, *Zheng* (meaning syndrome or pattern) is the overall physiological and/or pathological pattern of the human body in response to a given internal and external condition, which usually is an abstraction of internal disharmony defined by a comprehensive analysis of the clinical symptoms and signs gathered by a practitioner using inspection, auscultation, olfaction, interrogation, and palpation of the pulses (*1*). Correctly identifying the *Zheng* is fundamental for the diagnosis and treatment of diseases. Moreover, *Zheng* has been historically applied as the key pathological principle guiding the prescription of herbal formulas (Figure 1).

A lack of research on *Zheng* has left us with little understanding of its underlying biology or the relationships between different *Zhengs*, diseases, and drugs. Moreover, there have been attempts to integrate *Zheng* differentiation with modern biomedical diagnostic methods, though these efforts have not achieved the desired results (*2*). Many well-known herbal recipes, such as Liu Wei Di Huang Wan and Jin Kui Shen Qi Wan, have long been used for the clinical treatment of *Zheng* disorders; however, *Zheng*-guided treatments are still scarce due to the lack of evidence-based interpretations of syndromes and treatment efficacies. Thus, investigating the biological basis of *Zhengs* from a molecular to systems level is important for advancing the identification and treatment of these syndromes, and for providing more objective and quantitative diagnostic criteria.

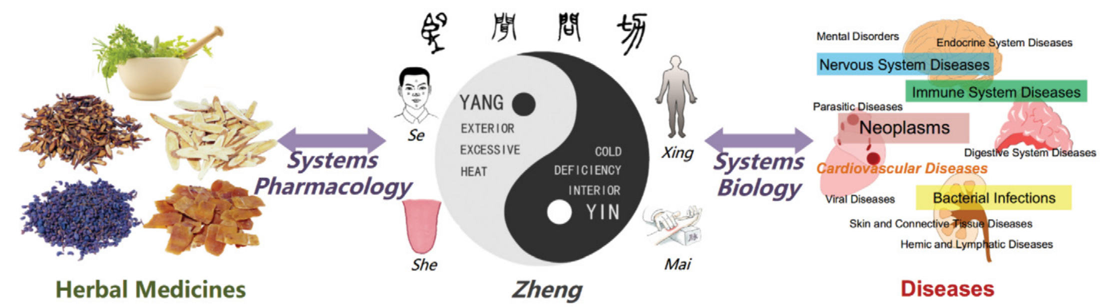
Zheng-guided disease research

In Western medicine, a disease is a particular abnormal and pathological condition that affects part or all of the human body and is often construed as a medical condition associated with specific symptoms. By contrast, *Zheng* puts forth a very different definition of a disease and encompasses all of the symptoms a patient presents.

Because of the highly interconnected nature of the human interactome, it is difficult to study different diseases at the molecular level completely independent of one another (*3*), and this issue also applies to *Zhengs*. Moreover, *Zhengs* are dynamic with changing boundaries, overlapping symptoms, and a multiscale nature, which makes them difficult to understand at a biological and mechanistic level. Thus, we propose that a comprehensive *Zheng* map be constructed that links together all the *Zhengs* based on their molecular and cellular relationships. Further, we suggest creating the "Zhengome" as a new 'omics field, in which a network is the basic research unit used to investigate the hierarchy present in the human body, from the molecular to the systems level. A comprehensive understanding of the Zhengome requires us to bring together multiple sources of evidence, from shared genes to protein-protein interactions, shared environmental factors, common treatments, and phenotypic and clinical manifestations, in order to capture the relationships between the different *Zhengs*.

Zheng uses the Yin-Yang, exterior-interior, cold-heat, and deficiency-excess definitions to describe patients' conditions, which are then managed by *Zheng*-specific recipes (Figure 1). Modern 'omics techniques combined with bioinformatics and bionetwork models through a systems biology approach have

FIGURE 1. Using systems pharmacology and systems biology approaches for understanding TCM Zheng can help bridge the gap between herbal medicines and diseases. Se, face color; Xing, body shape; She, tongue texture; Mai, pulse.



Materials that appear in this section were not reviewed or assessed by *Science* Editorial staff, but have been evaluated by an international editorial team consisting of experts in traditional medicine research.

School of Basic Medical Sciences, Beijing University of Chinese Medicine, Beijing, China
*Corresponding Author: xuanlong@bucm.edu.cn

been applied to investigate the differences between *Zhengs* and to identify novel biomarkers. For instance, rheumatoid arthritis (RA) patients differentiated on the basis of "hot" and "cold" *Zhengs* have been shown to be associated with different underlying genomic and metabolomic profiles, with the RA hot group showing more apoptotic activity than the cold group (*4*). Additionally, Li et al. used a network-based computational model to understand *Zheng* in the context of the neuro-endocrine-immune network and found that cold and hot *Zhengs* were closely related to a metabolism-immune imbalance (*5*). Wang and colleagues investigated the urine metabolome of patients with jaundice syndrome and its two subtypes of Yang Huang (acute) and Yin Huang (chronic), and identified several biomarker metabolites (*6*). However, most of the current studies have relied on only one or two approaches for molecular profiling and have lacked an efficient method to integrate data obtained at different 'omic levels. These studies also did not look at combining the analysis of molecular data with clinical variables, possibly missing an opportunity to generate more convincing conclusions. Considering the limitations of past studies, future efforts should integrate an analysis for all levels of 'omics (e.g., genomics, transcriptomics, epigenomics, and proteomics) data from a large number of patient samples for different *Zhengs* and include an investigation of the prognostic and therapeutic utilities of the data as a whole. Moreover, combining these molecular data with patients' clinical information could provide evidence-based theoretical interpretations for *Zhengs* and enable an assessment of *Zheng*-based therapeutic approaches.

Zhengs may change dynamically during disease progression. Differentiating the specific *Zheng* involved in each stage of a disease could provide valuable guidance for prescribing a dynamic therapeutic recipe. Using dynamic network modeling, a disease process can be conceptualized as spatio-temporal changes in network structures. The changes associated with a *Zheng* under dynamic therapy can be used to identify the key factors in the dynamic biological networks. Appropriate network perturbation models and subsequent robustness and topology analysis could help unveil potential disease-related genes or therapeutic targets involved in a disease's progression or evolution (*7*). The relationships between the different aspects of a disease (e.g., main symptoms versus complications) in a specific *Zheng* as well as the psychological, social, and even environmental factors should be taken into account during the modeling and simulation process in order to uncover the dynamic nature of complex diseases. Combining a Zhengome approach with dynamic modeling has the potential for establishing an accurate and quantitative *Zheng* research model, as well as for creating a new system for performing disease research.

Zheng-driven drug discovery

Despite considerable progress in genome, transcriptome, proteome, and metabolome-based high throughput screening methods and in rational drug design, drug discovery often encounters considerable costly failures that challenge the fidelity of the modern drug discovery system. *Zheng*-driven drug discovery has shown tremendous success for traditional drug discovery throughout Chinese medicine's history. However, since this concept is completely new to Western medicine, it is no easy task to incorporate *Zheng*-driven drug discovery into modern drug discovery workflows.

Here, we propose the "*Zheng* to TCM" and "TCM to *Zheng*" strategies within the framework of systems pharmacology to investigate biological systems and develop new therapeutics (Figure 2). The first strategy, *Zheng* to TCM, proposes developing a pipeline from *Zheng* diagnoses to TCM drugs, including differentiating *Zhengs*, identifying *Zheng*-related diseases and the associated genes and proteins, reverse targeting of drug effects, constructing and analyzing network/systems, and finally identifying effective herbal medicines (*8*). In effect, this strategy can be considered a reverse targeting and screening approach that is designed to uncover drugs from natural products that can target multiple *Zhengs* or related diseases. The goal of this method is to help researchers identify the active components within medicinal plants and multi-ingredient synergistic herbal formulas or drug combinations (*9*). In fact, this novel strategy has already been successfully applied in a *qi*-blood study, where we identified the active compounds in the *qi*-enriching and blood-tonifying herbs, their targets, and the corresponding pathways involved in the treatment of *qi* and blood deficiency syndromes (*8*).

The second strategy, TCM to *Zheng*, consists of a whole-system evaluation process starting with herbs or herbal formulas and culminating in identifying the *Zhengs*. This process includes the initial collection and classification of herbal medicines; screening the ingredients for absorption, distribution, metabolism, excretion, and toxicity (ADME/T); performing targeted drug screenings and tissue localization; constructing and analyzing networks; and finally identifying *Zhengs*/diseases (*10*). Using this strategy, it is possible to identify novel multitarget drugs in natural products (*11*). One particularly striking example is the systematic analysis of blood stasis and *qi* deficiency syndrome in coronary heart disease and the herbal drugs used to treat the syndromes. The results indicate that the herbs for eliminating blood stasis have pharmacological activity that acts to dilate blood vessel, improve the microcirculation, reduce blood viscosity, and regulate blood lipid, while *qi*-enhancing herbs have the potential for enhancing energy metabolism and anti-inflammatory activity (*12*). The TCM to *Zheng* strategy can also help to elucidate the pharmacological effectiveness of herbs and formulas.

In our ongoing work investigating Pi-deficiency syndrome (PDS) in the context of *Zheng*, we are analyzing patient samples using the sequencing alternative polyadenylation sites (SAPAS) method, RNA sequencing (*13*), lipid metabolomics, proteomics, and transcriptomics in order to decipher the pathogenesis and complex responses of the human body to PDS. From a drug development perspective, we plan to systematically investigate the *Si Jun Zi* decoction, a widely used herbal recipe for PDS, within the framework of the "TCM to *Zheng*" strategy, so as to understand why this recipe can regulate the immune response, stimulate blood circulation, and adjust gastrointestinal digestive functions. Despite the progress in *Zheng*-guided drug discovery, its future success requires the integration of multidisciplinary technologies, together with further innovations in these technologies, to facilitate the understanding of multifactorial diseases and the development of new therapies.

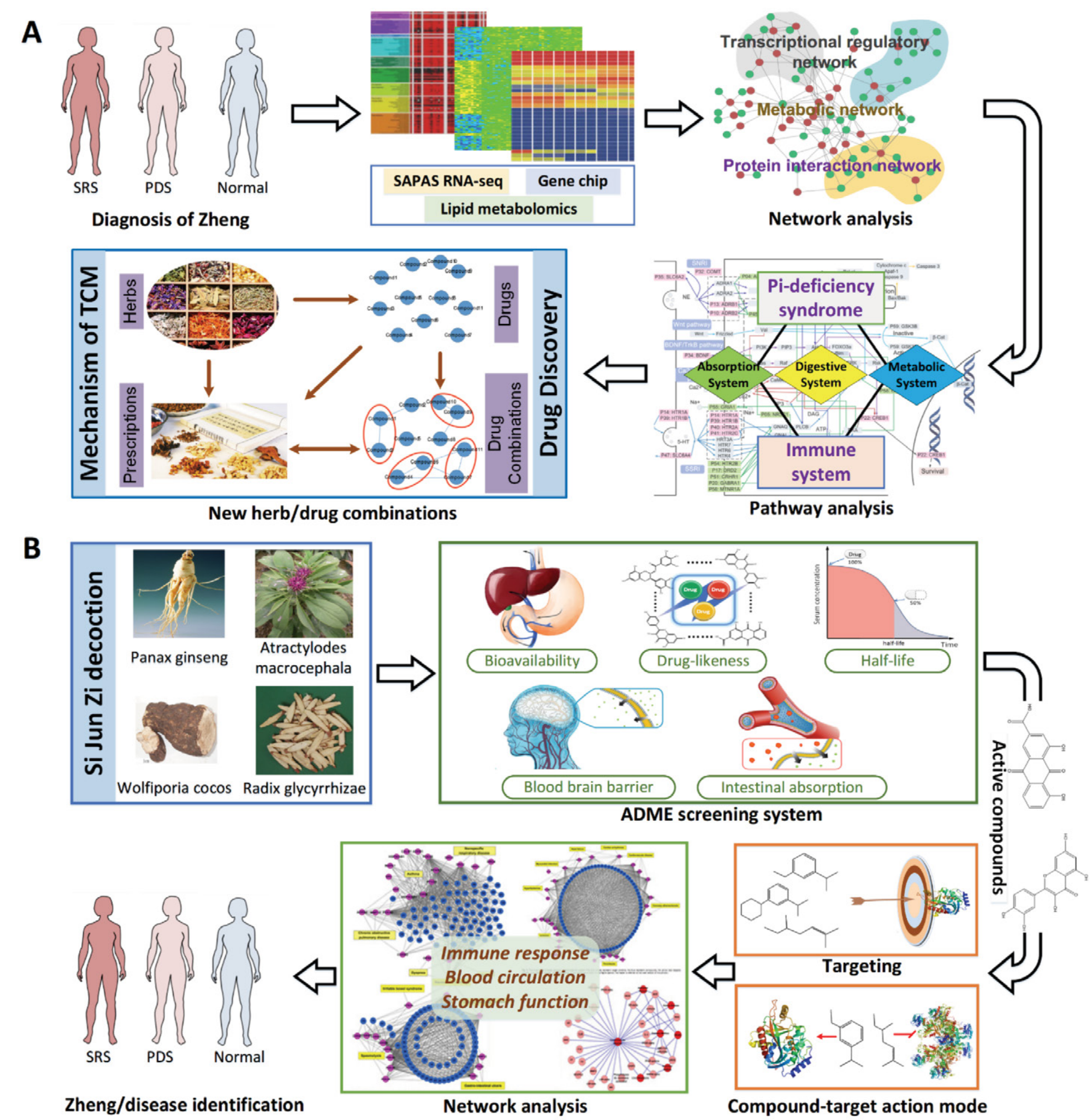


FIGURE 2. Zheng-driven drug discovery using Pi-deficiency syndrome and Si Jun Zi decoction as examples. (A) A "*Zheng* to TCM" procedure can build a pipeline from Pi-deficiency syndrome (PDS) to new effective herb/drug combinations by integrating 'omics data, network and pathway analysis, and pharmacokinetic evaluation technologies. SRS, Shi-Re syndrome, is a positive control for PDS. (B) An example of a "TCM to Zheng" strategy consisting of an entire evaluation process from Si Jun Zi decoction (SJZD) to PDS through a systems pharmacology strategy including text mining, ADME/T (absorption, distribution, metabolism, excretion, and toxicity) screening, target identification, and network analysis to reveal the underlying mechanisms of SJZD activity and build new diagnostic methods for PDS.

References

1. F. Cheung, *Nature* **480**, S82 (2011).
2. A. Lu, M. Jiang, C. Zhang, K. Chan, *J. Ethnopharmacol.* **141**, 549 (2012).
3. A. L. Barabasi, N. Gulbahce, J. Loscalzo, *Nat. Rev. Genet.* **12**, 56 (2011).
4. H. van Wietmarschen *et al., J. Clin. Rheumatol.* **15**, 330 (2009).
5. S. Li *et al., IET Syst. Biol.* **1**, 51 (2007).
6. X. Wang *et al., Mol. Cell. Proteomics* **11**, 370 (2012).
7. P. Csermely, T. Korcsmaros, H. J. M. Kiss, G. London, R. Nussinov, *Pharmacol. Therapeut.* **138**, 333 (2013).
8. J. Liu *et al., Evid. Based Compl. Alt. Med.* **2013**, 938764 (2013).
9. P. Li *et al., J. Ethnopharmacol.* **151**, 93 (2014).
10. C. Huang *et al., Brief. Bioinform.* **15**, 710 (2014).
11. C. Zheng *et al., Mol. Diversity* **18**, 621 (2014).
12. W. Zhou, Y. Wang, *J. Ethnopharmacol.* **151**, 66 (2014).
13. Y. G. Fu *et al., Genome Res.* **21**, 741 (2011).

Produced by the *Science*/AAAS Custom Publishing Office

Integrated network-based medicine: The role of traditional Chinese medicine in developing a new generation of medicine

Authors:
Elaine Lai-Han Leung, Vincent Kam-Wai Wong, Zhi-Hong Jiang, Ting Li, Liang Liu*

According to the philosophy of traditional Chinese medicine (TCM), health is the state of harmony between individual internal physiological networks (IPNs) and external environmental networks (EENs). Aberrant interactions between and within these networks cause complex diseases. TCM is grounded in these holistic principles, integrating philosophies from art and science; it stresses the maintenance of balance, or homeostasis, between the systems of the body and nature.

We believe that this kind of network-based holistic approach to medicine offers a useful counterpoint to today's biological reductionism-based thinking. We champion integrated network-based medicine (INBM) which takes a systems approach to understanding the individual's body as a whole, as opposed to relying on discrete components such as gene mutations, in order to explain illness (*1*). Built on the principles of IPNs and EENs, INBM offers a comprehensive medical system that integrates fundamental theories, diagnostic methods, and therapeutics based on a holistic and dynamic network-based approach.

The INBM system

Reductionist approaches to medicine, such as phenotype-based and target-based biomedicine (TBBM), are limited by their failure to consider the interactive nature of the human body and its environment. TBBM often views a disease as a tissue/organ-based condition that presents a single target for treatment, such as the elimination of a pathogen or the suppression of a disease-associated molecular target. This narrow focus can miss a broader range of pathogens and targets within the physiological and environmental networks. It may also overlook potentially useful global network effects that an intervention may have. For example, metformin was originally regarded solely as an anti-diabetic drug that inhibits the mitochondrial respiratory chain and activates the 5′ adenosine monophosphate-activated protein kinase pathway, resulting in inhibition of gluconeogenesis and the lowering blood glucose levels (*2*). Recently, a novel anti-cancer effect of metformin was identified by studying the overall impact of drug on the glucose metabolic network (*3*). This has raised the possibility that the drug will have new therapeutic uses (*4*). Efforts to focus on a single target can also have deleterious effects on the body's overall system. An example is indomethacin, a conventional Western-medicine drug. Indomethicin exerts an anti-inflammatory effect by inhibiting prostaglandin E2 (PGE2) synthesis (*5*), but this suppression of PGE2 also affects a receptor for mucus secretion, leading to gastric mucosa damage (*6*, *7*). A holistic view of the body's network of connections will anticipate such positive and negative impacts of medical treatments.

INBM requires rigorous conceptual design and practical implementation, and TCM has many principles and resources to help achieve this. These include "pattern differentiation in diagnosis and treatment of diseases," which can be regarded as a basic principle for individualized INBM (*8*). The "three m's" of Chinese herbal medicine (CHM) provides another example: these are "multi-chemical components," "multi-pharmacological effects," and "multi-action targets and pathways." The complex herbal formulae of CHM are intended to holistically modulate a person's physiological/pathological networks and, in developing new drug combinations, the "three m's" offer a useful optimization tool (*9*).

Figure 1 illustrates how the "three m's" approach to the

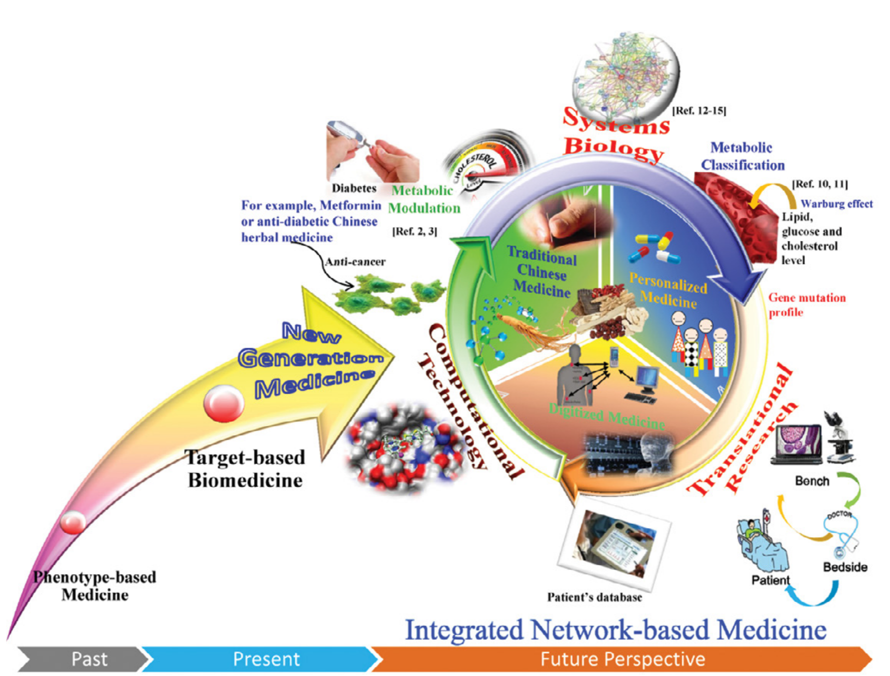


FIGURE 1. How integrated network-based medicine (INBM) works. TCM, traditional Chinese medicine.

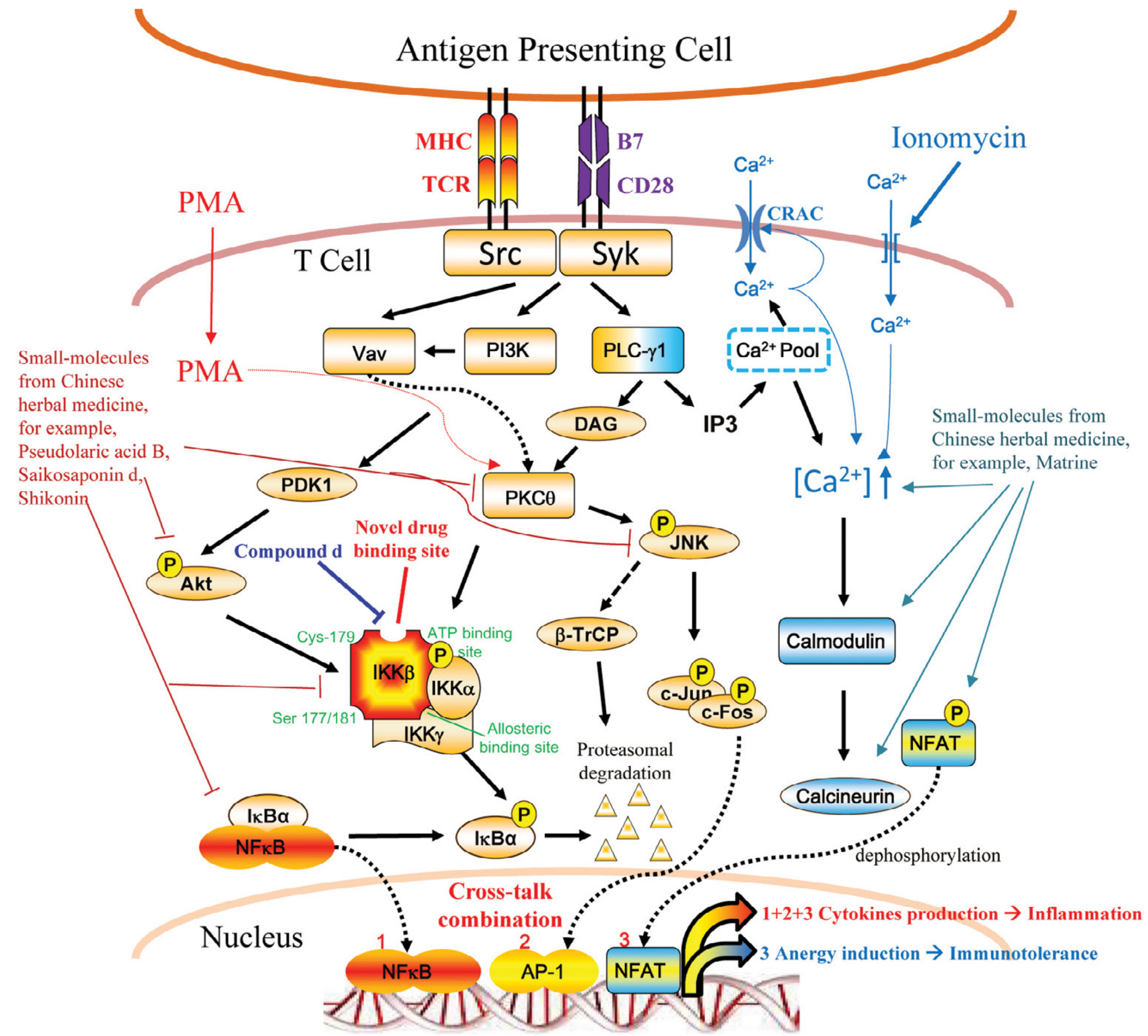


FIGURE 2. The impact of Chinese herbal medicine (CHM) components on signal transduction pathways involved in immune activation. PMA, phorbol myristate acetate; MHC, major histocompatibility complex; TCR, T cell receptor; CRAC, calcium release-activated channels. Ca2+, calcium ions; B7, B cell activation antigen B7; CD28, Cluster of Differentiation 28; Src, proto-oncogene tyrosine-protein kinase Src; Syk, spleen tyrosine kinase; PI3K, phosphatidylinositol-4,5-bisphosphate 3-kinase; PLC-γ1, phospholipase C γ1; DAG, dimeric acidic glycoprotein (clusterin); IP3, inositol trisphosphate; PDK1, pyruvate dehydrogenase lipoamide kinase 1; PKCθ, Protein kinase C θ; JNK, c-Jun N-terminal kinase; Akt, serine/threonine-specific protein kinase, also known as protein kinase B; β-TrCP, β-transducin repeat-containing protein; IKKβ, IκB kinase β; IKKα, IκB kinase α; IKKγ, IκB kinase γ; NFAT, nuclear factor of activated T cells; IκBα, IκB kinase α; NFκB, nuclear factor-κB; AP-1; activator protein 1.

State Key Laboratory of Quality Research in Chinese Medicine, Macau University of Science and Technology, Macau, China
*Corresponding Author: lliu@must.edu.mo

development of CHMs can be combined with digitalized medicine advances such as systems biology techniques, computational technologies, and translational research to provide the foundation for a "personalized" INBM strategy. Our proposal for a new personalized approach to cancer treatment provides just one example of the use of CHM for a network-based treatment. We have recently investigated two CHMs, *Panax ginseng* and Rhizoma Coptidis, and found that they inhibit cancer growth, prompting us to investigate the effects of these herbs on cancer cell metabolism. Using profiling methods such as liquid chromatography, mass spectrometry, and nuclear magnetic resonance, we found alterations to the glucose and fatty acid metabolic pathways at a network level (*10, 11*). We therefore speculate that metabolic biomarkers could be used to identify subgroups of patients with lipid- and glucose-related metabolic disorders. These patients are likely to benefit most from the herbs' active compounds. The approach could be further refined by the application of 'omics technologies to optimize the synergistic effect of the herbal remedies (*12–15*). With clinicians and basic researchers working together to create a database of personal therapeutic responses, continual improvements to herbal formulations would become possible.

From TBBM to INBM

How do we turn the INBM system from an idea into a practice? One major step forward would be the systematic quantitative and qualitative analysis of individual CHM components. Such a database would help define parameters for the combination treatments. One requirement is to identify, monitor, and control the major metabolic pathways, as altering a key molecular switch could have unintended, amplified effects on the entire network. A number of studies have begun to identify the specific network effects of various CHM-extracts or CHM-derived chemicals, including red yeast rice, *Tripterygium wilfordii* Hook. F., *Ganoderma lucidum*, San Miao Wan, arsenic sulfide, astragaloside IV, *Artemisia capillaris* Thunb, Radix Angelica Sinensis, Realgar-Indigo naturalis formula, and quinolones (*11–16*). In another example from our own work, we recently found that an active component from *Ampelopsis grossedentata* targets a novel drug-binding site on IKK-β kinase. The use of this ingredient in combination with dexamethasone suppresses inflammation in mice and reduces one of dexamethasone's side-effects: shrinkage of the thymus (*17*). We have also investigated matrine, an alkaloid found in Radix Sophorae Flavescentis. This small molecule can activate the Ca^{2+}-NFAT (nuclear factor of activated T cells) pathway, resulting in anergy (immune unresponsiveness), suggesting that it may be useful for treating autoimmune disorders (*18*).

Further investigation of network connections revealed that the CHM components PAB, saikosaponin d, and shikonin inhibit IKK-β and NFκB pathways simultaneously, while NFAT activation can be triggered by matrine. As shown in Figure 2, the overall impact is to trigger immunotolerance (*19–21*). A number of other studies have also demonstrated network-based effects in inflammatory diseases and cancer with CHM compounds such as PHY906 (*22*), curcumin (*23*), and berberine (*24*).

In conclusion, the development of INBM will enhance our medical and health care system, and TCM has an important role to play in building the foundation for the approach. The route from TBBM to INBM has obstacles, from unraveling the crosstalk of multiple molecular pathways to understanding CHM's network effects, to digitalizing the large amounts of data. Nevertheless, TCM—and our ancestors' wisdom—offers us a blueprint for establishing and implementing an INBM system for the betterment of humankind.

References

1. H. J. Federoff, L. O. Gostin, *JAMA* **302**, 994 (2009).
2. G. Rena, E. R. Pearson, K. Sakamoto, *Diabetologia* **56**, 1898 (2013).
3. D. Li, *J. Diabetes* **3**, 320 (2011).
4. R. J. Dowling, M. Zakikhani, I. G. Fantus, M. Pollak, N. Sonenberg, *Cancer Res.* **67**, 10804 (2007).
5. S. G. Rhind, G. A. Gannon, M. Suzui, R. J. Shephard, P. N. Shek, *Am. J. Physiol.* **276**, R1496 (1999).
6. P. Kalinski, *J. Immunol.* **188**, 21 (2012).
7. T. J. Weber, T. J. Monks, S. S. Lau, *Am. J. Physiol.* **273**, F507 (1997).
8. W. L. Hsiao, L. Liu, *Planta Med.* **76**, 1118 (2010).
9. C. T. Keith, A. A. Borisy, B. R. Stockwell, *Nat. Rev. Drug Discov.* **4**, 71 (2005).
10. R. J. DeBerardinis, C. B. Thompson, *Cell* **148**, 1132 (2012).
11. M. Journoud, P. J. Jones, *Life Sci.* **74**, 2675 (2004).
12. F. F. Costa, *Drug Discov. Today* **19**, 433 (2014).
13. J. van der Greef *et al.*, *Planta Med.* **76**, 2036 (2010).
14. Q. Y. Zhang *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 3378 (2009).
15. J. Zhao, P. Jiang, W. Zhang, *Brief. Bioinform.* **11**, 417 (2010).
16. J. Sendzik, M. Shakibaei, M. Schafer-Korting, H. Lode, R. Stahlmann, *Int. J. Antimicrob. Ag.* **35**, 366 (2010).
17. Z. Q. Liu *et al.*, *J. Pharmacol. Sci.* **99**, 381 (2005).
18. T. Li *et al.*, *Biol. Pharm. Bull.* **33**, 40 (2010).
19. T. Li *et al.*, *J. Cell. Biochem.* **108**, 87 (2009).
20. T. Li, F. Yan, R. Wang, H. Zhou, L. Liu, *Evid. Based Compl. Alt. Med.* **2013**, 379536 (2013).
21. V. K. Wong, H. Zhou, S. S. Cheung, T. Li, L. Liu, *J. Cell. Biochem.* **107**, 303 (2009).
22. W. Lam *et al.*, *Sci. Transl. Med.* **2**, 45ra59 (2010).
23. A. Koeberle, O. Werz, *Drug Discov. Today*, Epub ahead of print (2014), doi: 10.1016/j.drudis.2014.08.006.
24. Z. Li, Y. N. Geng, J. D. Jiang, W. J. Kong, *Evid. Based Compl. Alt. Med.* **2014**, 289264.

The hunt for antifibrotic and profibrotic botanicals

Authors:
Qihe Xu[1*],
Yibin Feng[2],
Pierre Duez[3],
Bruce M. Hendry[1],
Peter J. Hylands[4]

The U.S. government estimates that 45% of deaths in the United States can be attributed to fibrotic diseases, which are characterized by tissue scarring and often lead to chronic organ failure (*1*). Over the past several decades, researchers have investigated the underlying mechanisms involved in fibrosis and successfully pinpointed a number of possible drug targets, such as molecular mediators and effector cells. Moreover, a number of exceedingly potent and selective compounds against such targets have been developed, although many have fallen short of expectations (*2*). For example, the only antifibrotic drug registered in Europe and the United States, pirfenidone, has shown beneficial effects in patients with idiopathic pulmonary fibrosis and fibrotic kidney diseases; however, evidence for its efficacy lies in modest functional improvements, although its clinical efficacy on fibrosis remains elusive (*3–5*). On the other hand, a number of herbal medicinal products, such as those used in traditional Chinese medicine (TCM), have been reported as modulators of fibrosis, but definitive, comprehensive scientific evidence of botanicals as safe and effective antifibrotic therapeutics is lacking.

FIGURE 1. Steps proposed for an efficacy-based drug development strategy, particularly well-suited for studying antifibrotic botanicals.

Botanicals: A double-edged sword

Botanicals are an important source of antifibrotic activities. For example, halofuginone, a derivative of febrifugine isolated from *Dichroa febrifuga* Lour., and curcumin from *Curcuma longa* L., are reportedly antifibrotic (*6–8*); silymarin, a standardized mixture of flavolignans from milk thistle [*Silybum marianum* (Linn.) Gaertn.], has been widely used as a hepatoprotective and antifibrotic agent in chronic liver diseases (*9, 10*). Fuzheng Huayu, a formula widely used in China to prevent and reverse hepatic fibrosis, has recently completed a Food and Drug Administration (FDA)-approved phase II trial in the United States (*11, 12*).

To discover and compare inflammation-independent antifibrotic activities, we have developed high throughput cellular models of fibrosis to visualize and quantify excessive accumulation of total collagens (a gold standard for clinical diagnosis of fibrosis) and the subsequent disruption of cell monolayers (resembling fibrosis-induced disruption of a tissue's architecture) (*13*). Using this in vitro platform, we have established the direct antifibrotic activities of five active compounds, 11 individual herbs, and 16 herbal formulae (*14, 15*).

We found that Fuzheng Huayu and *Salvia miltiorrhiza* Bunge (SMB) root, a main component of Fuzheng Huayu, display the most potent in vitro antifibrotic activities among all the formulae and herbs that were tested (*14*). Besides, in a recent systematic review on clinical treatment of chronic hepatitis B—which took into account 138 trials, 62 proprietary traditional drugs, and 16,393 patients—SMB and its extracts were pinpointed among the top five herbal entities reported to have the most potent antifibrotic activities (*16*).

In contrast, some botanicals are suspected of causing fibrosis. Herbs have been regularly reported as being associated with chronic liver damage, from Africa to Asia and across the world (*17–21*). In clinical reports from Beijing and Shanghai, for example, herbs accounted for 21%-53.6% of drug-induced liver injury (*18, 22, 23*). In one of these same studies, biopsy findings indicated that liver fibrosis is not uncommon in patients with herb-associated liver injury (*18*).

Herbs have also been reported to be associated with fibrosis of the heart, mesentery, and kidney (*24*). For example, mesenteric fibrosis has been associated with long-term consumption of formulae containing *Gardenia jasminoides* Ellis fruits in Japanese patients and renal fibrosis is now well known to be induced by some *Aristolochia* taxa and other species containing aristolochic acids (AAs) (*25–27*). Once reported for medicinal use across a number of different regions, AA-containing plants are now recognized as a worldwide health threat and banned in most Western countries due to their association with AA nephropathy (AAN), including Balkan endemic nephropathy, which results from consuming grains contaminated by *Aristolochia* seeds (*27*). In vitro and in vivo studies indicate that many other herbs are associated with renal fibrosis. Notable examples include *Dioscorea villosa*

Materials that appear in this section were not reviewed or assessed by *Science* Editorial staff, but have been evaluated by an international editorial team consisting of experts in traditional medicine research.

[1]Department of Renal Sciences, Division of Transplantation Immunology and Mucosal Biology, MRC Centre for Transplantation, King's College London, London, U.K.
[2]School of Chinese Medicine, The University of Hong Kong, Hong Kong SAR, China
[3]Department of Therapeutical Chemistry and Pharmacognosy, Université de Mons, Mons, Belgium
[4]Institute of Pharmaceutical Science, King's College London, London, U.K.
*Corresponding Author: qihe.xu@kcl.ac.uk

L. rhizome, an herb commonly used in Australia to treat symptoms of menopause and rheumatoid arthritis (*28*), and *Leonurus japonicus* Houtt., an herb commonly used in TCM for gynecology and obstetrics (*15*, *29*). Additionally, we previously found that ethanolic extracts from unprocessed main roots of *Aconitum carmichaeli* Debx. have potent in vitro profibrotic activities (*15*)—this is likely clinically relevant since consumption of *Aconitum* species is known to cause acute renal failure and has been recently linked to end-stage renal disease (ESRD) in a case report by the U.K. Medicines and Healthcare Products Regulatory Agency (*30*).

The impact of identifying and avoiding exposure to profibrotic botanicals is profound. For instance, about one-third of the Taiwanese population consumed AA-containing herbs between 1997 and 2003 (*31*), and AAN previously accounted for up to 10% of all ESRD in Taiwan (*32*). The banning of AA-containing herbs, together with other efforts such as public-awareness campaigns, education of patients, funding for research into chronic kidney disease, and provision of integrated care, has turned Taiwan into one of the few regions with retarded increase of ESRD incidence (*33*).

Moving forward

Due to the contradictory and complex roles botanicals play in fibrotic diseases, there is an urgent need for studies that investigate the efficacy, safety, and good practices for botanical-based remedies.

Since fibrotic diseases are multifactorial conditions and botanicals are typically multitarget entities, an efficacy-based strategy is particularly well-suited for studying antifibrotic botanicals (Figure 1).

Such a strategy is highly dependent on disease modeling. It is worth emphasizing that innovation is needed to develop high-quality in silico, in vitro, and in vivo models that can facilitate the investigation of antifibrotics and detect profibrotic activities.

Because evidence-based medicine is a relatively new concept in many countries (*34*), many clinical reports on herbal treatment of fibrotic diseases are criticized for poor quality. Diseases for which the literature has been recently reviewed include liver fibrosis (*35*, *36*), pulmonary fibrosis (*36*), multiple sclerosis (*36*), and adhesive small bowel obstruction (*37*). An efficacy-based strategy ultimately demands high-quality clinical trials to prove antifibrotic effects and invites interregional cooperation on pharmacovigilance of profibrotic botanicals, which is challenging due to the insidious nature of fibrosis and the variability in the distribution channels and legal status of botanicals across regions (*38*, *39*).

Finally, traditional use is only an indication but certainly not a proof of either safety or efficacy (*40*). To harness and understand botanicals both as potential antifibrotic therapeutics and for the prevention of fibrotic diseases, future research and innovation must focus on efficacy and safety, and must be built on and contribute to good practices, which we have recently defined at length (*41*). Development and refinement of good practices, however, can only be achieved with sustainable funding.

References

1. T. A. Wynn, *Nat. Rev. Immunol.* **4**, 583 (2004).
2. S. L. Friedman, D. Sheppard, J. S. Duffield, S. Violette, *Sci. Transl. Med.* **5**, 167sr1 (2013).
3. P. W. Noble *et al.*, *Lancet* **377**, 1760 (2011).
4. K. Sharma *et al.*, *J. Am. Soc. Nephrol.* **22**, 1144 (2011).
5. M. E. Cho, D. C. Smith, M. H. Branton, S. R. Penzak, J. B. Kopp, *Clin. J. Am. Soc. Nephrol.* **2**, 906 (2007).
6. M. Pines, I. Vlodavsky, A. Nagler, *Drug. Dev. Res.* **50,** 371 (2000).
7. D. Punithavathi, N. Venkatesan, M. Babu, *Br. J. Pharmacol.* **131**, 169 (2000).
8. R. Bruck *et al.*, *Liver Int.* **27**, 373 (2007).
9. N. D. Freedman *et al.*, *Aliment Pharmacol. Ther.* **33**, 127 (2011).
10. F. Wei *et al.*, *Eur. J. Clin. Microbiol. Infect. Dis.* **32**, 657 (2013).
11. P. Liu, *Chin. J. Integr. Med.* **18**, 398 (2012).
12. Z. Xu, *Nature* **480**, S90 (2011).
13. Q. Xu, J. T. Norman, S. Shrivastav, J. Lucio-Cazana, J. B. Kopp, *Am. J. Physiol. Renal Physiol.* **293**, F631 (2007).
14. Q. Hu *et al.*, *Nephrol. Dial. Transplant.* **24**, 3033 (2009).
15. Q. Xu *et al.*, in *Recent Advances in Theories and Practice of Chinese Medicine*, X. Kuang Ed, (Intech, Rijeka, 2012), p. 337.
16. T. Zhan, X. Wei, Z. Q. Chen, D. S. Wang, X. P. Dai, *J. Tradit. Chin. Med.* **31**, 288 (2011).
17. B. J. Auerbach *et al.*, *PLOS ONE* **7**, e41737 (2012).
18. R. T. Lai *et al.*, *Zhonghua Gan Zang Bing Za Zhi* **20**, 185 (2012).
19. J. B. Wang *et al.*, *PLOS ONE* **6**, e24498 (2011).
20. J. Y. Wang *et al.*, *Liver Int.* **34**, 583 (2014).
21. R. Teschke, J. Schulze, A. Schwarzenboeck, A. Eickhoff, C. Frenzel, *Eur. J. Gastroenterol. Hepatol.* **25**, 1093 (2013).
22. F. Q. Hou , T. L. Wang, X. Liu, N. Huo, G. Q. Wang, *Hepatogastroenterology* **57**, 554 (2010).
23. G. D. Zhou *et al.*, *Zhonghua Gan Zang Bing Za Zhi* **15**, 212 (2007).
24. D. H. Connor, K. Somers, A. M. Nelson, P. G. D'Arbela, R. Lukande, *Trop. Doct.* **42**, 206 (2012).
25. K. Hiramatsu *et al.*, *Aliment Pharmacol. Ther.* **36**, 575 (2012).
26. K. Nomura *et al.*, *Nihon Shokakibyo Gakkai Zasshi* **109**, 1567 (2012).
27. M. R. Gökmen *et al.*, *Ann. Intern. Med.* **158**, 469 (2013).
28. K. Wojcikowski, H. Wohlmuth, D. W. Johnson, G. Gobe, *Food Chem. Toxicol.* **46**, 3122 (2008).
29. R. Sun, X. Wu, J. Liu, L. Sun, L. Lv, *Pharmacology and Clinics of Chinese Materia Medica* **21**, 28 (2005).
30. MHRA warns of the dangers of taking unlicensed herbal medicines containing aconite. 2013, http://www.mhra.gov.uk/Safetyinformation/Generalsafety informationandadvice/Herbalmedicines/ Herbalsafetyupdates/Allherbalsafetyupdates/CON263932.
31. S. C. Hsieh, I. H. Lin, W. L. Tseng, C. H. Lee, J. D. Wang, *Chin. Med.* **3**, 13 (2008).
32. F. L. Lin Wu *et al.*, *Nephron. Clin. Pract.* **120**, c215 (2012).
33. V. Jha *et al.*, *Lancet* **382**, 260 (2013).
34. J. Wang, *Lancet* **375**, 532 (2010).
35. F. Cheung *et al.*, *Chin. Med.* **7**, 5 (2012).
36. J. Liu, in *The Fundamentals of Fibrosis, and the Prevention and Treatment of Fibrosis in Traditional Chinese Medicine*, J. Niu, C. Ben, Eds. (People's Health Press, Beijing, 2008), p. 31.
37. T. Suo *et al.*, *Cochrane Database Syst. Rev.* **5**, CD008836 (2012).
38. EMA, EMA publication EMA/322570/2011 Rev. 3, 2013, http://www.ema.europa.eu/docs/en_GB/document_library/Report/2011/05/WC500106706.pdf.
39. T. P. Fan *et al.*, *J. Ethnopharmacol.* **140**, 568 (2012).
40. Q. Xu *et al.*, *BMC Complement Altern. Med.* **13**, 132 (2013).
41. H. Uzuner *et al.*, *J. Ethnopharmacol.* **140**, 458 (2012).

Acknowledgments

The authors thank Kidney Research UK and the European Commission for their funding support, and Professor Chih-Wei Yang (Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Taiwan) and Professor Joëlle Nortier (Erasme Hospital, Université Libre de Bruxelles, Brussels, Belgium) for their expert advice in the preparation of this manuscript.

i-Needle: Detecting the biological mechanisms of acupuncture

Authors:
Christine Nardini[1,2*†],
Sandro Carrara[3*†],
Yuanhua Liu[2],
Valentina Devescovi[2],
Youtao Lu[2],
Xiaoyuan Zhou[2]

A long standing obstacle to the (full) integration and acceptance of acupuncture in conventional medicine lies in the difficulty of reconciling traditionally defined categories (acupoints, meridians, and energy flow or *qi*) with anatomical structures and biochemical pathways. Additionally, a unified scientific theory to explain the diverse effects of acupuncture (from pain control to immunomodulation) is lacking, despite important advances in the association of purinergic signaling with the effects of acupuncture on pain control. As new technologies simultaneously offer enhanced capacities to explore breadth (using 'omics) and depth (using nanobiochips) of biochemical events, we propose the innovative conjugation of these approaches into an intelligent needle (*i*-needle) as a means to overcome the abovementioned limitations.

Acupuncture is being widely debated in the medical community as a potential alternative or complementary treatment for many diseases (*1*). There are numerous challenges to achieving a consensus over the use of acupuncture in a medical environment, including: filling the gap in knowledge about the underlying molecular mechanisms of acupuncture, and (re)interpreting traditional categories (such as acupoints, meridians, and *qi*) and therapeutic indications within an evidence-based medicine framework. Important questions aimed at increasing our understanding of the molecular effects of needle stimulation have been posed, mostly regarding pain control (*2*), functional recovery of tissue (*3*), and immunomodulation (*4*), with remarkable work done as to the correlation of pain control with purinergic signaling (*5*, *6*). Using 'omics-based technology and network representations, researchers have successfully mapped the molecular underpinnings of traditional categories (*7*). More generally, the holistic method used in acupuncture, which has long been difficult to reconcile with the scientific reductionist viewpoint, has recently been found to be compatible with a systems biology approach (*8*).

'Omics-based techniques are diverse and allow for the screening of targets from nucleic acids (DNA-sequencing, RNA-sequencing) to proteins and metabolites (mass spectrometry/liquid chromatography, nuclear magnetic resonance) and their heterogeneous interactions (chromatin immunoprecipitation-sequencing), to name just the major technologies. Recently, whole new areas of exploration have been opened with metagenomics and metatranscriptomics where the host-microbiome relationship can be analyzed systemically and in situ. Further, rapidly decreasing costs are permitting researchers to prefigure relatively high spatial (different body regions and tissues) and temporal resolution. Here, we propose to integrate such highly resolved molecular, temporal, and spatial data to reveal the molecular signaling pathways that flow from the tip of the needle to the disease/injury site.

Understanding the biochemical signaling pathway that the mechanical rotation of an acupuncture needle sets into motion (*9*) is an important starting point. Mechanosensing and mechanotransduction are widespread in biology with well-assessed relevance in embryonic development, i.e., type 1 epithelial-mesenchymal transition (EMT) (*10*). Their roles, however, have not been well explored under the broader definition of EMT (*11*)—which includes events such as wound healing (type 2 EMT) and cancer (type 3 EMT)—despite promising therapeutic results when mechanical stimulation is locally applied (*12*). Acupuncture needle stimulation (*9*) and low level laser therapy (*13*) are among the triggers that have been shown to initiate a series of synergistic events, including calcium waves, ATP fluxes (purinergic signaling), and changes in reactive oxygen and nitrogen species concentration, known to initiate healing (*14, 15*). The homeostatic effects of type 2 EMT include local changes in purinergic signaling, inflammation control, regeneration, and remodeling at the site of injury. By contrast, acupuncture is recommended for systemic diseases like rheumatoid arthritis (*1*) and is thought to act in a more global fashion.

Using the framework we propose here, we can investigate the long range, systemic effects of mechanotransduction by building on what has already been reported about the wound healing process, including the presence of peripheral markers of EMT (*16*).

To explore the long range effects of acupuncture, multiomic analysis of molecular events—occurring proximally (acupoint), distally from the stimulation point (target organ), and systemically (blood and gastrointestinal microbiome)—can be used to construct a spatial analysis (*17*). This information can then be enriched with data about the temporal onset of early gene expression, in addition to later time points (Figure 1A) to construct a systems biology view (network) of the biochemical events.

To build such networks and identify new targets for diagnosis and therapy, computational analysis must bring together the different 'omics approaches (Figure 1B), coupled with the requisite temporal and spatial resolution of the data (*19*). This type of network approach can identify the most important molecules from the thousands to tens-of-thousands of interactions and hundreds-to-thousands of molecules analyzed, also taking into account distal factors that might play a role in causing or modulating the pathologies.

Furthermore, the identification of additional markers is made possible with a complementary approach to the high

[1]Lazzari, Bologna, Italy
[2]Group of Clinical Genomic Networks, Key Laboratory of Computational Biology, CAS-MPG Partner Institute for Computational Biology, Shanghai Institutes for Biological Sciences, Shanghai, China
[3]Bio/interfaces Group, Integrated System Laboratory, EPFL-École Polytechnique Fédérale de Lausanne, Switzerland.
[*]Corresponding Authors: christine.nardini.rsrc@gmail.com (C.N.) and sandro.carrara@epfl.ch (S.C.)
[†]These authors contributed equally to this work

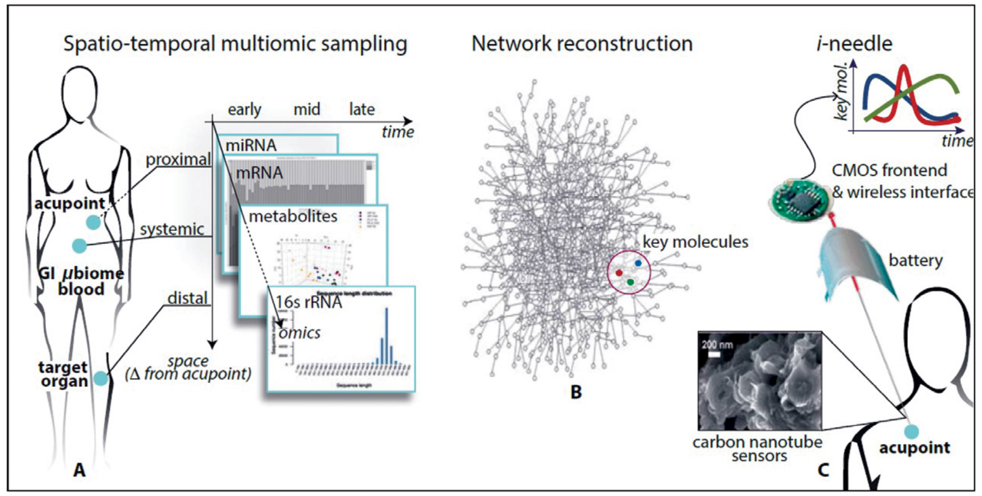


FIGURE 1. Elements of the *i*-needle. (A) A variety of 'omics techniques can be used to monitor molecular progress across body sites and over time. The pictured model shows the spatiotemporal multi-'omic sampling of the molecular flow of events over the therapy's delivery, from early molecular activation at the acupoint to the peripheral bloodstream and gastrointestinal (GI) microbiome (μbiome) to ultimately reach the target organ. **(B)** Multi-'omic systems biology enables the identification of a network of events, allowing interpretation of acupuncture in terms of a biochemical signaling flow that alters the whole system (body/patient). Network analysis and simulations allow identification of molecules that can be monitored as markers of the progress of the therapy (*18*). **(C)** Diagram showing carbon nanotube (CNT)-based sensor integration to continuously monitor the therapy-induced biochemical progression. Sensors are mounted on an energy-autonomous device that is able to transmit information remotely and in real-time.

throughput and low sensitivity of these 'omics analyses. This can be imagined in the form of a nanobiochip that is the size and shape of an acupuncture needle (hence, an "intelligent" needle or *i*-needle) (Figure 1C).

Toward this end, we recently created a proof-of-principle miniaturized platform, integrating revolutionary carbon nanotubes and nanographite petals, which can monitor five endogenous human metabolites using highly sensitive and selective nanobiosensors (*20*). The electronics needed to acquire and transfer the detected signal have already been sufficiently miniaturized (*21*) and can be powered by ultrathin polymer-based batteries (*22*) currently available on the market and able to meet the energy demands of the proposed *i*-needle (~80–130 μAh).

The challenge for the realization of the *i*-needle has already moved from the miniaturization to the integration step (*23*). Progress has already been made, based on recent reports of the measurement and transmission of temperature, pH, and endogenous metabolite data using single-platform enzyme-carbon nanotube hybrid sensors (*24, 25*) .

Conclusions

Overall, it is our hope that this research can provide a more unified approach to understanding the complex nature of patient responses to acupuncture—including effects as diverse as the control of pain, degeneration, and inflammation—and to addressing fundamental issues in acupuncture treatment, such as the frequency of delivery, developing more precise therapeutic indications, and establishing proper "dosage" guidelines. These steps will undoubtedly encourage acceptance of acupuncture as a complementary and/or alternative personalized treatment, with important application in a wide variety of areas including pain control, and degenerative and chronic inflammatory diseases, among others.

References

1. WHO, *Acupuncture: review and analysis of reports on controlled clinical trials*. (WHO, Geneva, Switzerland, 2002), http://apps.who.int/iris/handle/10665/42414.
2. G. Burnstock, *Med. Hypotheses* **73**, 470 (2009).
3. Y. Ding *et al.*, *BMC Neurosci*. **10**, 35 (2009).
4. Y. Liu *et al.*, *PLOS ONE* **8**, e51573 (2013).
5. N. Azorin *et al.*, *Exp. Dermatol.* **20**, 401 (2011).
6. W. Z. Tu *et al.*, *Neurochem. Int.* **60**, 379 (2012).
7. H. van Wietmarschen *et al.*, *J. Clin. Rheumatol.* **15**, 330 (2009).
8. J. Jia *et al.*, *J. Ethnopharmacol.* **140**, 594 (2012).
9. H. M. Langevin, N. A. Bouffard, G. J. Badger, D. L. Churchill, A. K. Howe, *J. Cell. Physiol.* **207**, 767 (2006).
10. A. Mammoto, T. Mammoto, D. E. Ingber, *J. Cell Sci.* **125**, 3061 (2012).
11. R. Kalluri, R. A. Weinberg, *J. Clin. Invest.* **119**, 1420 (2009).
12. V. W. Wong, S. Akaishi, M. T. Longaker, G. C. Gurtner, *J. Invest. Dermatol.* **131**, 2186 (2011).
13. E. Takai, M. Tsukimoto, H. Harada, S. Kojima, *Radiat. Res.* **175**, 358 (2011).
14. J. V. Cordeiro, A. Jacinto, *Nat. Rev. Mol. Cell. Biol*. **14**, 249 (2014).
15. G. C. Gurtner, S. Werner, Y. Barrandon, M. T. Longaker, *Nature* **453**, 314 (2008).
16. M. Mildner *et al.*, *PLOS ONE* **8**, e60103 (2013).
17. S. E. Winter, C. A. Lopez, A. J. Baumler, *EMBO Rep.* **14**, 319 (2013).
18. P. Tieri, X. Zhou, L. Zhu, C. Nardini, *Front. Cell Dev. Biol.*, **2**, Epub before print (2014), doi: 10.3389/fcell.2014.00059.
19. B. Kholodenko, M. B. Yaffe, W. Kolch, *Sci. Signal.* **5**, re1 (2012).
20. I. Taurino *et al.*, *Nano Letters* **14**, 6 (2014).
21. J. Yoo, L. Yan, S. Lee, Y. Kim, H.-J. Yoo, *IEEE Journal of Solid-State Circuits* **45**, 178-188 (2010).
22. H. Nishide, K. Oyaizu, *Science* **319**, 737 (2008).
23. T. E. Kazior, *Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences* **372**, 20130105 (2014).
24. A. Cavallini, C. Baj-Rossi, S. Ghoreishizadeh, G. De Micheli, S. Carrara, *Biomedical Circuits and Systems Conference (BioCAS), 2012 IEEE*, pp. 124 (2012).
25. S. Carrara, A. Cavallini, S. Ghoreishizadeh, J. Olivo, G. De Micheli, in *IEEE Sensors conference*, (2012), pp. 28–31.

Purinergic signaling in acupuncture

Author:
Geoffrey Burnstock

The proposed role of purinergic signaling in the physiological basis of acupuncture was first presented in 2009. Data showing that ATP is released from keratinocytes and other skin cells during acupuncture treatments lends weigh to this hypothesis. ATP in turn activates P2X3 receptors on the sensory nerves in the skin, which then transmit those messages to motor neurons in the brain stem that control autonomic functions and modulate nociceptive activities. Here, we review and describe the recent evidence for purinergic signaling underlying acupuncture effects and propose ways to further test this hypothesis.

Introduction

It has been well established that adenosine 5′-triphosphate (ATP) is an intracellular energy source in cellular biochemistry. In 1970, Burnstock et al. suggested that ATP acted as a nonadrenergic, noncholinergic neurotransmitter in the gut (*1*), and in 1972 he named the extracellular actions of ATP, "purinergic signaling" (since ATP is a purine nucleotide), and formulated the purinergic signaling hypothesis (*2*).

In 2009, Burnstock proposed that purinergic signaling could be involved in the physiological mechanisms mediating acupuncture effects. This hypothesis suggested that mechanical deformation of the skin by needles or application of heat or electrical current leads to the release of large amounts of ATP from keratinocytes, fibroblasts, and other cell types in skin (Figure 1). The released ATP then activates P2X3 ion channel receptors on sensory nerves within the skin and tongue that transmit messages via sensory ganglia and the spinal cord to the brain stem and hypothalamus. These brain regions contain motor neurons that control autonomic functions, including cardiovascular, gastrointestinal, respiratory, and urinogenital activities—common targets of acupuncture treatments. These sensory neuron messages also modulate the pathways that lead to centers in the cortex responsible for conscious awareness of pain and other central nervous system activities, including sleep regulation (3). A number of subsequent studies have been published that also implicate purinergic signaling in various aspects of acupuncture, detailed below.

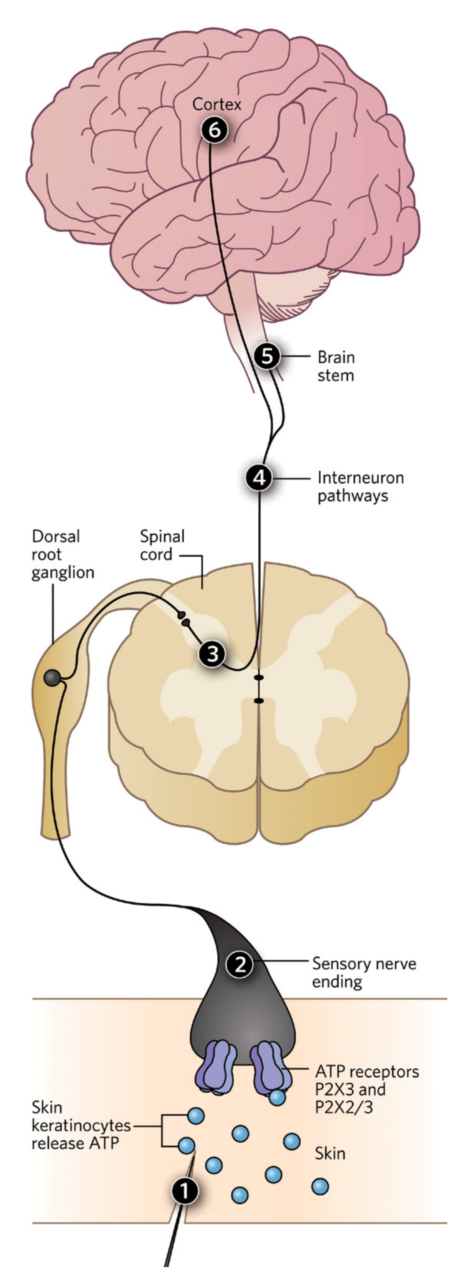


FIGURE 1. Acupuncture and purinergic signaling. Insertion and twisting of the needles employed in acupuncture mechanically deforms the skin, leading to the release of ATP by skin keratinocytes (*1*). ATP binds to specific receptors located on sensory nerve endings in the skin, P2X3 and P2X2/3 (*2*). The signaling message is then relayed via dorsal root ganglia to the spinal cord (*3*) and subsequently through interneuronal pathways (*4*) to the brain stem (*5*) that contains motor neurons, which control the functions of gut, lung, heart, arteries, and reproductive organs—all major targets for acupuncture. Signals also travel to the pain centers of the cortex, delivering a message to inhibit pain (*6*). (Reproduced with permission from reference *36*.)

Supporting evidence for the hypothesis

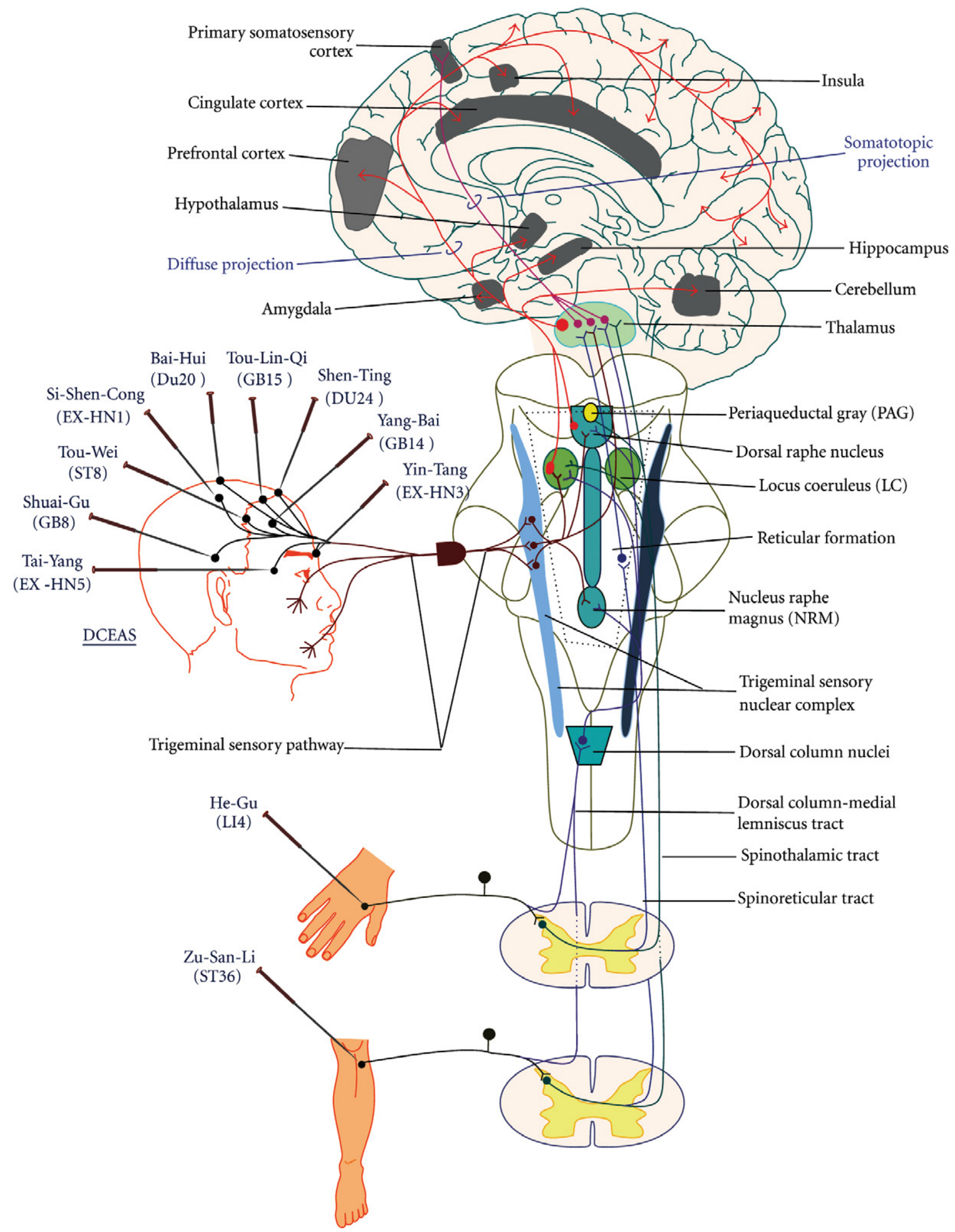
Studies that have established the components involved in the purinergic signaling pathway include: (i) release of ATP (in response to mechanical or chemical stimulation)

Materials that appear in this section were not reviewed or assessed by *Science* Editorial staff, but have been evaluated by an international editorial team consisting of experts in traditional medicine research.

Autonomic Neuroscience Centre, University College Medical School, London, UK, and Department of Pharmacology and Therapeutics, The University of Melbourne, Melbourne, Australia
g.burnstock@ucl.ac.uk

Produced by the *Science*/AAAS Custom Publishing Office

FIGURE 2. Schematic illustration of the central neural pathways that carry afferent (sensory) neural impulses following acupuncture treatment from various parts of the body. Brain areas that commonly respond in neuroimaging studies to acupuncture stimulation are indicated with gray shadow. DCEAS: dense cranial electroacupuncture stimulation. (Reproduced from *28*, with permission from Hindawi Publishing Corporation.)



from keratinocytes (*4–6*) and possibly from Merkel cells, which contain high levels of ATP (*7, 8*); ATP has also been shown to be released from keratinocytes upon heating (*9*); (ii) immunohistochemical data demonstrating the presence of P2X3 receptors on sensory nerve fibers in the skin (*10–12*) and tongue (*13*); (iii) in an isolated tongue/lingual nerve preparation, mechanical activation of the tongue with De Frey hairs was shown to result in a discharge in the lingual sensory nerve fibers that was mimicked by ATP activation and blocked by P2X3 receptor antagonists (*14*); and (iv) both presynaptic inhibition via adenosine A_1 and P2Y receptors, and enhancement via P2X and A_{2A} receptors at synapses in the central nervous system have been reported (*15*).

Subsequent papers have built upon and extended evidence in support of purinergic signaling underlying acupuncture effects. Several studies have associated the skin cells affected by acupuncture techniques with purinergic signaling. For example, ATP has been shown to be released from human keratinocytes in response to mechanical stimulation by hypo-osmotic shock (*16*), as well as from keratinocytes in response to heat (*17*). Additionally, mast cells, which accumulate around the acupuncture needles, also release ATP in response to mechanical stimulation (*18*). Another skin cell type, human subcutaneous fibroblasts, can also release ATP in response to bradykinin and histamine (*19, 20*). Tsutsumi et al. demonstrated that mechanical stimulation can evoke the propagation of calcium waves between human keratinocytes, induced by ATP and activation of $P2Y_2$ receptors (*21, 22*), which is consistent with the earlier results from Koizumi et al. (*5*). Tuina (traditional therapeutic massage) and moxibustion (a traditional Chinese medicine therapy using a moxa, often made from dried mugwort, either used as a fluff or processed into a cigar-shaped stick; it can be used indirectly, with acupuncture needles, or burned on to the patient's skin) may also act via the purinergic signaling pathway (*23*). Papers describing the release of ATP from human epidermal keratinocytes via connexin hemichannels and vesicles involving vesicular nucleotide transporter have recently been published (*24–26*). A 2010 study has claimed that adenosine, following breakdown of released ATP during acupuncture, can act as a prejunctional inhibitor of neurotransmission via A_1 receptors, resulting in anti-nociceptive actions (*27*). Valuable reviews are available describing the neural pathways from different skin regions to structures in the brain stem and higher brain centers. These pathways are important because different acupuncture sites may activate different neural pathways impinging on specific nuclei in the brain stem that control autonomic functions potentially modulated by acupuncture (Figure 2) (*28, 29*).

Purinergic signaling and electroacupuncture

Electroacupuncture is a form of acupuncture where a small electric current is passed between pairs of acupuncture needles. This is thought to augment traditional acupuncture and is believed to be particularly helpful in treating pain.

The supraspinal antinociception effect of electroacupuncture has been associated with P2X3 receptor activation in the midbrain periaqueductal gray region (*30*). Moreover, the analgesic effect of electroacupuncture on chronic neuropathic pain has been shown to be mediated by P2X3 receptors in rat dorsal root ganglion neurons (*31*). Following these studies, electroacupuncture was shown to result in a reduced expression of P2X3 and P2X2 receptors in the dorsal root ganglion of rats with chronic neuropathic pain (*32*) and visceral hypersensitivity (*33*). Electroacupuncture at *He-Mu* points can also reduce P2X4 receptor expression in colon and spinal cord in visceral hypersensitivity (*34*). Moreover, in a review by Lin et al., the neuroprotective effects of acupuncture were reported to act via increasing brain derived neurotrophic factor (BDNF) expression via stimulation of ATP (*35*).

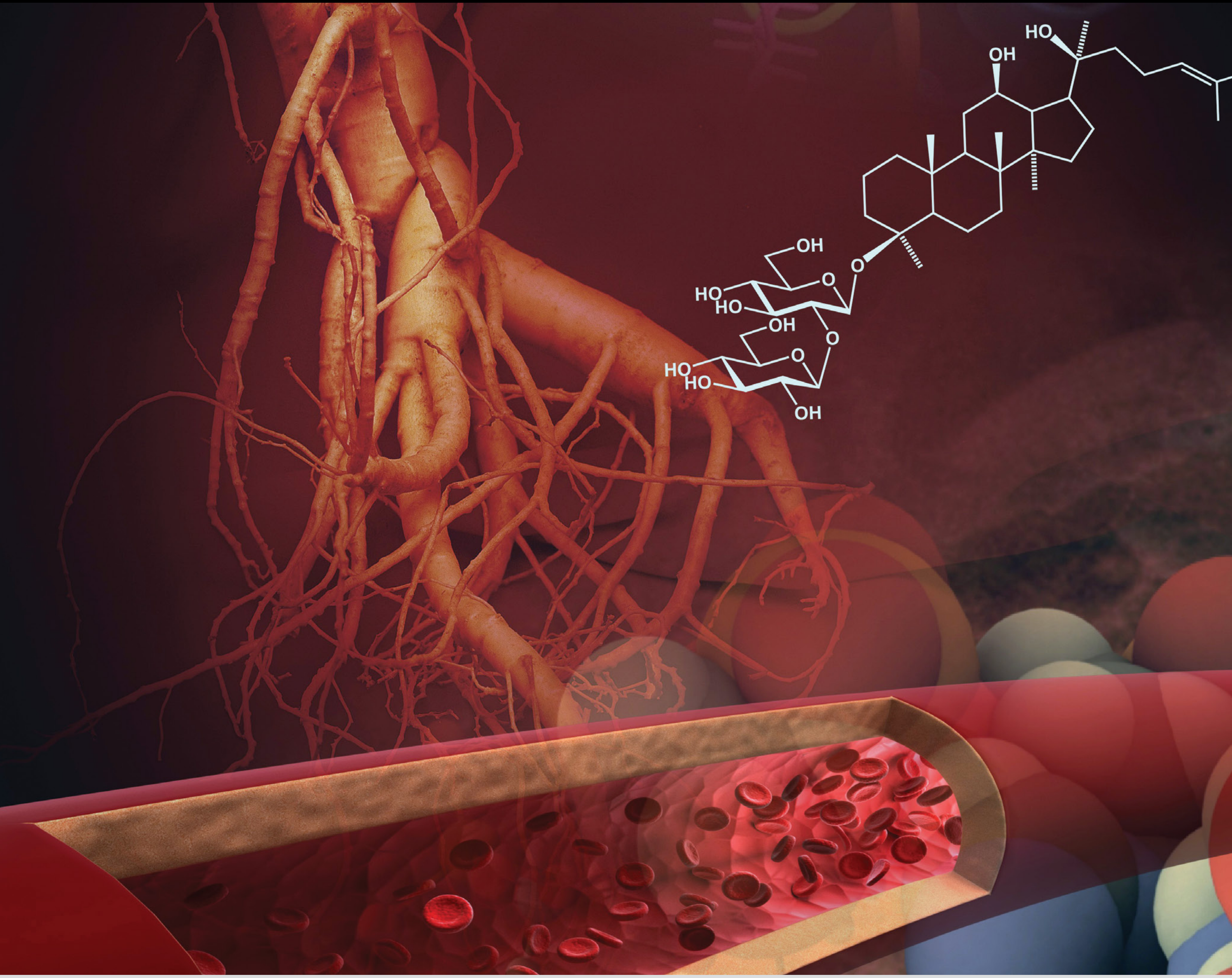
Conclusions

Evidence in support of the hypothesis of purinergic signaling mediating the physiological mechanisms underlying acupuncture effects has been accumulating over recent years. To help further test this hypothesis, I propose that experienced acupuncturists focus on acupuncture sites that induce effects that can be quantified, such as an increase or decrease in heart rate or blood pressure, and identify specific neurons that are activated in the brain using noninvasive scanning techniques. If acupuncture-induced effects can be identified and quantified, researchers could then test whether ATP mimicks the responses and if P2X3 receptor antagonists block the effects. Moreover, we suggest that researchers conduct experiments recording responses from sensory neurons in the skin and tongue in animal models and distinguish between low-threshold fibers involved in acupuncture and high-threshold fibers that mediate nociception, as well as recordings from the motor nerves in the brainstem responsible for autonomic functions.

References

1. G. Burnstock, G. Campbell, D. Satchell, A. Smythe, *Br. J. Pharmacol.* **40**, 668 (1970).
2. G. Burnstock, *Pharmacol. Rev.* **24**, 509 (1972).
3. G. Burnstock, *Med. Hypotheses* **73**, 470 (2009).
4. N. Mizumoto, M. E. Mummert, D. Shalhevet, A. Takashima, *J. Invest. Dermatol.* **121**, 1066 (2003).
5. S. Koizumi *et al.*, *Biochem. J.* **380**, 329 (2004).
6. H. E. Burrell *et al.*, *J. Biol. Chem.* **280**, 29667 (2005).
7. R. Crowe, M. Whitear, *Cell Tissue Res.* **190**, 273 (1978).
8. M. Silberstein, *Med. Hypotheses* **75**, 272 (2010).
9. S. Mandadi *et al.*, *Pflugers Arch.* **458**, 1093 (2009).
10. E. J. Bradbury, G. Burnstock, S. B. McMahon, *Mol. Cell. Neurosci.* **12**, 256 (1998).
11. G. Burnstock, *Br. J. Anaesth.* **84**, 476 (2000).
12. M. Taylor, J. C. Peleshok, A. Ribeiro-da-Silva, *J. Comp. Neurol.* **514**, 555 (2009).
13. X. Bo *et al.*, *Neuroreport* **10**, 1107 (1999).
14. W. Rong, G. Burnstock, K. M. Spyer, *J. Physiol.* **524**, 891 (2000).
15. G. Burnstock, *Physiol. Rev.* **87**, 659 (2007).
16. N. Azorin *et al.*, *Exp. Dermatol.* **20**, 401 (2011).
17. J. R. Gifford, C. Heal, J. Bridges, S. Goldthorpe, G. W. Mack, *J. Physiol.* **590**, 6403 (2012).
18. L. Wang *et al.*, *Evid. Based Complement. Alternat. Med.* **2013**, 350949 (2013).
19. R. Pinheiro *et al.*, *Cell Commun. Signal.* **11**, 70 (2013).
20. R. Pinheiro *et al.*, *J. Biol. Chem.* **288**, 27571 (2013).
21. M. Tsutsumi *et al.*, *Cell Tissue Res.* **338**, 99 (2009).
22. M. Tsutsumi *et al.*, *Skin Res. Technol.* **16**, 146 (2010).
23. L. Fan, L. M. Yin, *J. Acupunct. Tuina Sci.* **12**, 125 (2014).
24. T. P. Barr *et al.*, *PLOS ONE* **8**, e56744 (2013).
25. T. Takahashi *et al.*, *J. Invest. Dermatol.* **133**, 2407 (2013).
26. K. Inoue *et al.*, *J. Invest. Dermatol.* **134**, 1465 (2014).
27. N. Goldman *et al.*, *Nat. Neurosci.* **13**, 883 (2010).
28. Z. J. Zhang, X. M. Wang, G. M. McAlonan, *Evid. Based Complement. Alternat. Med.* **2012**, 429412 (2012).
29. Q. Q. Li *et al.*, *Evid. Based Complement. Alternat. Med.* **2013**, 267959 (2013).
30. Z. Xiao *et al.*, *Brain Res.* **1330**, 31 (2010).
31. W. Z. Tu *et al.*, *Neurochem. Int.* **60**, 379 (2012).
32. R. D. Cheng *et al.*, *Chin. J. Integr. Med.* **19**, 374 (2013).
33. Z. Wang *et al.*, *Neural Regen. Res.* **8**, 802 (2013).
34. X. Guo *et al.*, *Neural Regen. Res.* **8**, 2069 (2013).
35. D. Lin *et al.*, *Int. J. Mol. Sci.* **15**, 3234 (2014).
36. G. Burnstock, *The Scientist* **25**, 24 (2011).

Produced by the *Science*/AAAS Custom Publishing Office



The American Association for the Advancement of Science
1200 New York Avenue NW
Washington, DC 20005

Science
AAAS