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The handle http://hdl.handle.net/1887/19086 holds various files of this Leiden University dissertation.

Author: Hu, Chiuxiu
Title: Systems biology for evaluating system-based medicine
Issue Date: 2012-06-14

Chapter 1

General introduction and scope

General introduction

Life-style related risk factors have reached epidemic proportions worldwide and have been leading causes of morbidity and mortality in modernized societies. An early stage of these life-style related medical disorders is metabolic syndrome, which is characterized by a group of at least 3 metabolic risk factors in an individual person out of the following conditions: (1) abdominal obesity (excessive fat tissue in and around the abdomen), (2) atherogenic dyslipidemia (blood fat disorders — high triacylglycerides (TG), low level of high density lipoprotein cholesterol (HDL-C) and high level of low density lipoprotein cholesterol (LDL-C) — that foster plaque build-ups in artery walls), (3) elevated blood pressure, (4) insulin resistance or glucose intolerance (the body can't properly use insulin or blood sugar), (5) a prothrombotic state (*e.g.* high fibrinogen or plasminogen activator inhibitor–1 in the blood) and (6) a proinflammatory state (*e.g.* elevated C-reactive protein in the blood). Each of these disorders is by itself a risk factor associated with other diseases. In combination, however, these disorders dramatically boost the chances of developing cardiovascular disease and diabetes. The current situation of the global epidemics of these disorders and their associated diseases has provided adequate reasons for identifying and treating subjects with metabolic syndrome. An important factor in metabolic syndrome that has attracted a strong focal point of research is associated with atherogenic dyslipidemia as lipid-lowering therapies are believed to be key in improvement of global health care. Given the huge market opportunity of medicine addressing this condition, many pharmaceutical industries have medicine related to lipid disorders as an important component of drug discovery efforts. As a consequence, a remarkable increase in research spending and individual efforts has been put on the development of therapeutic targets and new drugs since the second half of the 20th century.

However, in the recent decade the output in terms of innovative products of the pharmaceutical industry is going down and the costs of the development process is rapidly increasing. There seems to be a limit in terms of opportunity from the target-driven paradigm of drug development. The innovation gap that has become apparent in pharmaceutical industry is not only an economic issue, but the quality of life of the consumers is another more important reason for new innovation needs. About 90% of the drugs work only for 30-40 % of the patients and this implies that a considerable number of patients get side-effects without any benefits. The paradigm of 1 disease – 1 target – 1 drug in other words "one size fits all" is coming to an end. The rise of system thinking and systems biology as a tool in biomedical research has opened new ways to understand biological living systems. Instead of focusing on a single target it has become apparent that understanding the self-organization of an organism is key to address the issues of health and disease. Furthermore the concept of disease management has come under critical review as for Health Care prevention is becoming a rapidly increasing topic on the agenda worldwide. Prevention however, relates directly to support the healthy system to maintain a healthy condition, so increasing the resilience of a system in view of possible future disease states is becoming a new field of attention, so-called salutogenesis. Instead of fighting against a disease as illustrated in the names of many drugs containing elements such as "anti-, blocking- and inhibitor", a switch is needed to supportive therapies. Systems theory outlines that the scale and the complexity of

the problem and the solution should match and for that reason an intervention with a single chemical compound has limited value for addressing a complex regulatory network that is out of balance. Combination therapy addressing multiple targets is more towards this goal and the intervention has some resemblance with playing a piano by touching elegantly several keys in a soft manner creating wonderful melodies versus hammering hard on just a single one. It becomes clear that in acute stages of disease the current concept works well, but it is very limited in chronic diseases or health promotion strategies. Designing combination therapy from scratch is not an easy task as we understand still too little of the complex biological living system. The growing change in awareness in the medical field that things need to be changed, the economic needs as the current system cannot be controlled from the cost perspective in the near future but above all that another paradigm needs to prevail in our Health Care system, opens up the combination of different perspectives available on a global level. Herbal medicine has been important as long as mankind existed but the importance has been down played by the successes of modern Western medicine.[1] The new insights into a systems-approach urge new studies on herbal medicine to reveal the multi-target synergetic effects which have hardly been used in modern drug discovery.[2] Moreover the favorable side-effect spectrum of herbal medicine and the origin of development based on health promotion in a personalized manner addresses exactly what is needed in our current societies. For this reason in the present thesis we have used a systems biology platform to undertake scientific studies to obtain a better understanding of Chinese herbal medicine formulas and to strengthen our understanding of regulatory mechanisms in humans. Moreover bridging Western and Chinese Medicine brings the body-mind split as being present in Western medical sciences since Descartes under renewed attention. This is crucial as for diagnostic purposes describing a patient only partially will never yield a good monitoring and evaluation opportunity let alone a good matching therapy.

In this thesis, we focus on both chemical drugs and herbal medicine to study the effects on lipid metabolic disorders in animal models at an early stage of obesity and type II diabetes mellitus. The aim is to get a better insight into the working mechanisms of both approaches and to see how they affect biological systems.

Systems biology

The reductionistic approach has been successfully used in understanding smaller and simpler units in living system and is often based on the principles that a complex biological system can be studied as isolated parts (*e.g.* cells, tissues and organism). As such, little is known on the comprehensive concepts to understand how and why the components function the way they do.[3] The reason being that new properties emerge at higher levels of organization and this cannot be deduced by studying the parts only.

In the recent decade, the tremendous breakthroughs in instrumentation, bioinformatics tools and analytical methodology have enabled the paradigm shift from the identification of individual components to study the interaction of these components in living systems.[4] This holistic view is required for a shift in understanding the intricate networks of interactions quantitatively.[5] Systems biology possesses such a system-wide perspective that focuses on the quantitative study of the behaviors and relationships of all the elements in a particular

functional biological system.[6] It is a biology-based cross-disciplinary study that requires the integration of biology, medicine, mathematics and chemistry together with biostatistics and bioinformatics to convert complex and diverse datasets into useful knowledge.[5,7] In order to identify biomarkers of health/disease and of drug/nutritional effects, it often requires parallel analyses of mRNA, proteins and metabolites from complex samples to link processes in cells, tissues and organisms to each other.[8] Figure 1 shows the flow chart of the systems biology approach as applied in life science research.
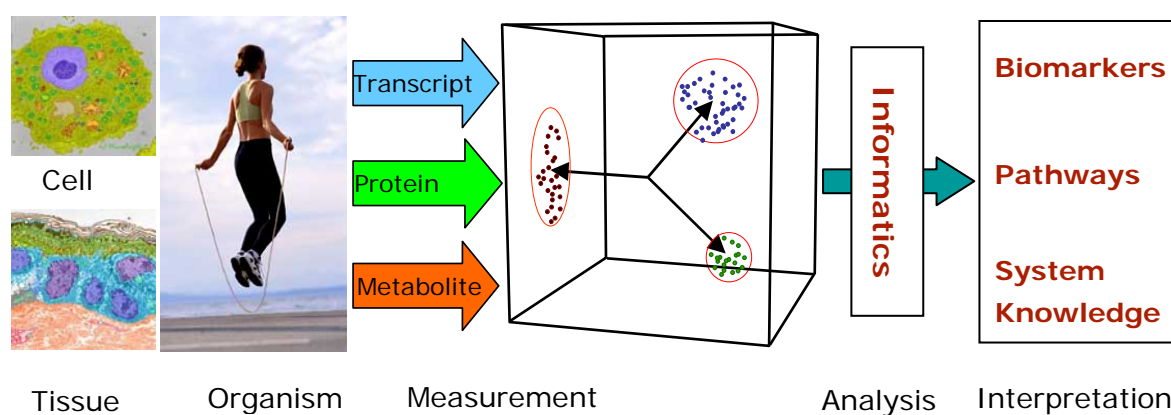
Figure 1. Flow chart of the systems biology approach as applied in life science research. Systems biology studies a biological system as an integrated system of genetic, protein, metabolite, cellular and pathway events that are in flux and interdependent, aiming at integration of data into information and eventually knowledge at the system level.

Philosophy of Chinese Medicine

Chinese Medicine has been practiced over thousands of years ago, and over time, a range of traditional practices based on experiences and clinical observations were gathered and developed into classical theories of Chinese Medicine in old classical Chinese texts. A representative masterpiece is *Huangdi Neijing* (Yellow Emperor's Inner Canon), the earliest classical Chinese medical text that is still considered essential in the practices of modern Chinese Medicine. This book explores many key principles of Chinese Medicine including Yin-yang, the Five Phases, Zang-Fu organs, etc. Influenced by the culture of Taoism, Buddhism and Confucianism, the theories of Chinese Medicine hold the belief that the world inside the body has an intimate connection with the world outside the body and the understanding of the human body should be based on the holistic understanding of the universe.[9] Under this cultural background, Chinese Medicine follows a holistic health care philosophy (*i.e.* looking at the behavior of the system as a whole) together with unique features including opposing principles, synergistic and combinatorial (*e.g.* using specific component(s) of herbal medicine in rational combinations) effects as well as personalized therapy. In Chinese Medicine, a diseased condition is considered a dysbalance of Yin-yang in the body. The treatment starts with diagnosis of the entire system, and then corrects the pathological changes through readjusting the functions of the Zang-fu organs. Differing from Western Medicine, Chinese Medicine is based on the principle of multiple component – multiple target interactions. It aims to use therapies in an effort to maintain/improve health

and treat disease and has become a useful model for scientific therapies and is practiced in many parts of the world. However, the approach of Chinese Medicine is (still) very much like a black box when it concerns the biochemical level. For example, the outputs (*e.g.* physical symptoms and signs) and inputs (*e.g.* pathogenic factors, herbs, diet, treatment, etc.) are observed without knowing the interaction mechanisms occurring inside the biological system. A systems biology approach applied in combination with advanced analytical tools opens up a unique and novel opportunity to study these complex natural products and their effects in biological systems.[10,11]

Metabolomics

In the recent two decades, great achievements in profound investigations on gene, mRNA and protein and metabolite levels in health, disease and drug therapy have been witnessed.[12] The fields of genomics, transcriptomics have become well-established and extensively developed with a large number of cutting-edge equipment available. Proteomics and metabolomics are still hampered by analytical challenges based on the large diversity and/or low concentrations to be measured. These studies have emphasized the need for research of new efficacy biomarkers in clinical trials. A shift in focus from a single biomarker towards "multiple" biomarkers to be used for monitoring, differential diagnosis and risk assessment" is observed. However, most of the research studies are often cell-based and as a consequence limited with respect to understanding the organism or tissue level comprising large numbers of molecules participating in the biological processes.
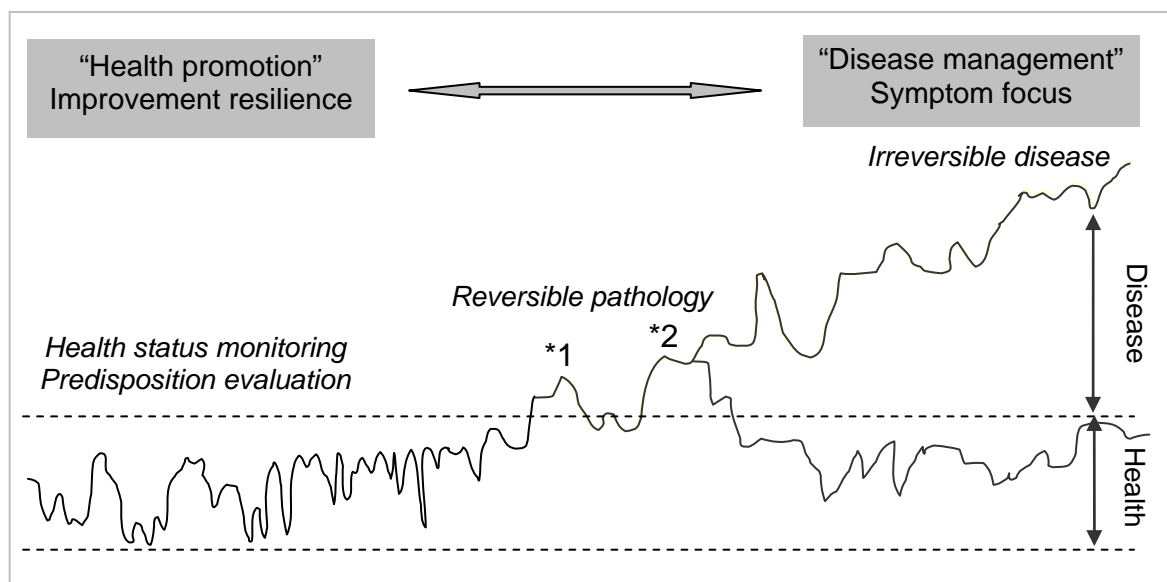
Figure 2. A schematic diagram of the development of disease from healthy (homeostasis) via sub-optimal health and ultimately to a disease state. (*1) represents a reductionist intervention that can be applied to reduce the given symptom; and (*2) represents a system intervention that can allow the system to regain its resilience. Traditionally the focus in Western Medicine is on disease management while Chinese Medicine has its focus on health promotion.

The response of organisms to biological stimuli by measuring changes at the level of metabolites found in biological fluids and tissues, can probe the effects of diet, nutrition, drugs and disease. Metabolomics technologies play a key role as the information is close to the phenotypic level. It enables the identification and quantification of small molecules in biological systems to monitor the systemic changes through time in complex multi-cellular systems when exposed to biological stimuli or genetic manipulation.[13] Such methodology is an essential part for systems biology, since it opens the option for an integrated view of biochemistry in complex organisms.[13] Actually, metabolomics-based systems biology has been an emerging field in the life sciences and pharmaceutical research, as it provides a unique platform to improve both diagnosis and treatment of human diseases by generating a unified view on biology and medicine through the integration of Western and Eastern knowledge[11,14] (see Figure 2). This emerging field of metabolomics is also driving a revolution in the biochemistry, the physiology and nutritional aspects of lipids,[15] as lipids form one of the largest and most diverse group of metabolites. As lipids act among others as metabolic and energy storage units and are involved in membrane structure and scaffolding for membrane proteins,[16] they play a key role in a range of (patho)physiological processes.

Metabolomics-based systems biology approaches are essential to describe the changes from normality to dysfunction, see Figure 2, from a diagnostic perspective and to serve as indicators of for pharmacological responses to a therapeutic intervention.

Lipidomics

The crucial role of lipids in biological system has been demonstrated by a large number of life-style related risk factors/diseases (*e.g.* obesity, hypertension, cardiovascular disease and diabetes) that are associated with the disturbance of lipid metabolic enzymes and pathways.[17] However, lipids are known to be very diverse in structure. The traditional way of determining the molecular structures of individual lipid molecules in biological samples through pre-separation of lipids into different lipid fractions cannot keep pace with the new concept of understanding the specific role of lipids in (patho)physiology that was brought forth at the beginning of this century. This novel concept requires detailed characterizing of changes in lipid metabolites and their interactions in a systems-integrated context in order to build a comprehensive picture of lipid metabolic interconnections, discover new molecular species and determine how lipids modulate biological functions.[17] Such novel concept within the metabolomics field is called lipidomics, an emerging field that has proven to be promising in life sciences and pharmaceutical research with a variety of applications in drug and biomarker development.[17] Such systems-level based "lipidomics" may offer novel insights into the effects of diet, drugs, herbal medicine and disease.

While this system-based lipidomics approach has gained considerable momentum in the life sciences and biomedical research currently, it is still confronted with major challenges due to the complexity of lipids and the diversity of the samples. The major challenges can be summarized as:
- advanced technical platforms are required that enable the comprehensive quantitative and qualitative analysis of all lipids present in cells, body fluids, tissues and such,

    preferably in a single measurement
- complexity of data generation (study design) and difficulty of integration of heterogeneous data
- bioinformatics tools that enable adequate biological interpretation of complex data

Scope of the thesis

With these challenges as described above in mind, the studies described in this thesis were aimed to investigate the feasibility of a systems-biology approach in multi-target therapies towards lipid metabolism and metabolic syndrome related risk factors/disease in (transgenic) animal models. The risk factors/disease comprised obesity and diabetes. As primary targets in the metabolomics approach, lipids were selected due to their crucial roles in biological systems. For this, adequate analytical tools are required to characterize and quantify numerous lipids (of different type) in complex matrices such as plasma and tissues from animal models, as detected in a response to (herbal) drug treatment.

Outline of the thesis

In **Chapter 2** the challenges in the comprehensive analysis of lipids are discussed and the present status of analytical strategies in lipidomics and their representative applications in diseased biomarker discovery are summarized. In addition, a particular example of multivariate statistical analysis is given to illustrate the powerful capacity of bioinformatics tools in adequately interpreting complex data in research field of lipidomics.

The development of a novel and fully validated method for the profiling of numerous (~ 150) lipids in plasma is described in **Chapter 3**. This analytical platform is capable in delivering detailed information to the understanding of the effects of intervention by both Western and Chinese Medicine on plasma lipid metabolism in various disease models. The capacities of the platform in searching of potential lipid biomarkers in relation to disease prevention and health promotion are challenged and evaluated throughout the subsequent research studies in the thesis.

In **Chapter 4** the analytical lipidomics platform is applied to study plasma samples from mice on high-fat diet with or without treatment with rimonabant, the first selective cannabinoid-1 (CB1) receptor blocker that has proven to be effective in food intake reduction, long-term maintained body weight loss and cardiovascular risk factor improvement in obesity subjects. After intensive validation, the analytical lipid platform was also used for the analysis of lipids in liver tissues of the mice. A result in this chapter both plasma and hepatic lipidomics response to rimonabant treatment in an animal model were statistically evaluated and biological interpreted.

The result of a study with the analytical lipidomics platform as applied to plasma and liver samples from mice on high-fat diet with or without a multi-target herbal formula is subject matter of **Chapter 5**. In this study, research questions to be answered were (1) Does SUB885C exhibit treatment effects on regulation of body weight and lipid-related metabolic parameters during early stage obesity? (2) Is SUB885C induced regulation of lipids and

lipoprotein profiles correlated with cholesteryl ester transfer protein? and (3) What is the activity of SUB885C in CB1 receptor binding affinity and lipogenesis?

In **Chapter 6** the analytical lipidomics platform was applied to evaluate 9-week treatment effects of metformin and different qualities of the well-known multi-component herb *Panax ginsengs* on the regulation of glycaemic control, lipid-related metabolic parameters and plasma lipid metabolism in rats with type 2 diabetes mellitus (T2DM). The research questions discussed in this chapter are related to (1) does *Panax ginseng* root show bioactivities in treatment of T2DM in terms of regulation of glycamemic, plasma biochemical parameters and plasma lipid metabolism? (2) Does the quality based on the culture time of the ginseng roots correlate with its bioactivity in treatment of T2DM? and (3) Does *Panax ginseng* play a similar role as metformin on glycaemic control in T2DM?

The outcomes of the different studies in the thesis are discussed in the context of the current different perspectives in Western and Chinese Medicine and the bridge that is formed when a systems view is adapted.

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