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REVIEW ARTICLE

Systems biology in heart diseases

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Abstract

Systems biology based on integrative computational analysis and high technology is in a position to construct networks, to study the interactions between molecular components and to develop models of cardiac function and anatomy. Clinical cardiology gets an integrated picture of parameters that are addressed to ventricular and vessel mechanics, cardiac metabolism and electrical activation. The achievement of clinical objectives is based on the interaction between modern technology and clinical phenotype. In this review the need for more sophisticated realization of the structure and function of the cardiovascular system is emphasized while the incorporation of the systems biology concept in predicting clinical phenotypes is a promising strategy that optimize diagnosis and treatment in cardiovascular disease. Hippokratia 2010; 14 (1): 10-16

Key words: systems biology, cardiac models, network biology, clinical phenotypes

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In the latter part of the last century, biology was driven by reductionist concepts that concerned with information originating from the interactions of genes and molecules. A novel conceptual idea developed over the last few years with the purpose of understanding biological processes which were not explained fully by the properties of genes and molecules. Both evolutionary theories over many decades and systems biology recently have transformed the consensus of biology.

Systems biology (SB) is a new discipline which tries to explain the biological phenomenon not only with delineation of the function of genes and molecules but by reconstructing biochemical supramolecular networks that represent various cellular functions. Systems biology concern with biochemical networks is focused on their functional status and on the nature of the links connecting the molecules of the network[1]. This concept considers the biological networks as 'systems' of cooperating and interacting components with complex behaviors.

This novel approach is transforming the way of thinking in research, biology and disease. Network modeling could explain cardiovascular metabolism and thus design new diagnostic and therapeutic tools. Also the SB perspective could help to predict and treat complex diseases such as heart failure, metabolic syndrome and diabetes mellitus.

Biological components and systems analysis

In the second half of the previous century after the discovery of the DNA construction, attention focused on explaining the cellular function and the chemical composition of isolated biological components (gene, protein, pathways). This reductionist tendency was increased with the advent of genomics and proteomics. The DNA sequences in a large number of organisms are known but their function at the moment is incomplete. The proteomic technology is important in predicting the activation of some particular genes but remains still in its infancy.

The recent advent of integrative analysis of the different gene products, the new experimental technologies, and in particular the notion that the cells are organized in systems has forced biologists to give special attention to the systems properties. This shift of focus from individual cellular components to systems properties and tissue organization is an inexorable process that eventually would apply to the study of tissue and organ functions. The appearance of novel systems properties in cellular, tissue or organ level, are considered properties emerging from the complex metabolic and regulatory networks of tissues and organs and are not properties of distinct cellular components.

There is a hierarchical coordination that in steps or levels involves genes, genetic products, cellular functions, tissues and organs. There is not a simple relationship between genes but rich biological networks that transform genetic potential into phenotypic reality. The coordinated function at each level between the different components produces a kind of circuit. The term "genetic circuit" was used to designate a collection of different gene products executing a particular cellular function[1]. Genetic circuits are responsible for information processing, metabolism, cellular function and evolution. Therefore the integrated functions of many genetic circuits form the basic material for cellular function and tissue as well as organ coordinated operation.

In a similar way with the molecular biology and the complex metabolic and regulatory networks, the scientific pursuit can extend to tissue and organ functional

coordination in the different levels of the hierarchical ladder. The advances in bioinformatics and clinical phenotype make plausible this kind of research and application[2-4].

The proceeding steps of systems biology

The process leading to the emergence of SB is a new paradigm of holistic decoding of biological systems function and consists of four principal steps[1]: a) Enumeration of biological components participating in the process including the plurality of –omics (Genome, Transcriptome, Proteome, Metabolome). b) Interaction diagrams between these biological components are depicted and the constructed cellular networks are demonstrated. Such examples of biological networks are gene and proteomic networks, immunological networks, and metabolomic and disease networks. c) Reconstructed networks are analyzed and the biological functions are predicted. d) The above emerging models should predict experimental outcomes and explore the phenotypic space.

The universal theories of networks by Barabasi et al emphasized that the cell functional organization is based on biological similar networks that comply with universal laws ruling complex systems[5-7].

It is likely that the SB from the level of genome-cell exploration would advance to the broader field of an organismic concept. This broader field of SB from the networks of cellular interactions would be directed to the diverse phenotypic space of tissues and organs. The previous mentioned hierarchical thinking starts from the DNA and continues to the systemic two-dimensional genome-scale stoichiometric matrix. The hierarchical structure extends from introns, exones, alleles and other components of DNA scale, through a network of chemical reactions, modules and pathways, to tissue functional organization and organ cooperation.

The cardiovascular (CV) disorders have the highest mortality rates in the world, change the quality of life and increase the economic burden of the society. Therefore there is great significance to understand the cellular and molecular alterations that influence the progression of cardiac pathologies. Systems biology is an important current area of biological research and the practical application of the accumulated molecular and cellular knowledge can give some answers to the field of cardiology. In this overview we summarize the most important areas of cardiac systems biology as a new approach to decode cardiovascular diseases. The tools of SB are ideal for analyzing the complex molecular programs involved in regulatory cardiac networks.

Systems biology components (-omics) in cardiology

The discipline of SB has evolved after progress was made in molecular biology, new technologies and understanding of complex metabolic and regulatory networks. Important studies have been directed at genome, proteome, transcriptome and metabolome connecting DNA with messenger RNA, intracellular proteins and metabolites. The accumulated and informative data should be integrated as the need has emerged for the construction of a functional cellular model[8].

Genomic studies produced an extensive list of genes connected with the heart in various physiological and disease states and in a variety of temporal stages and spatial locations. In some studies genes have been identified and expressed in different diseases encoding special proteins[9]. These genes were responsible for sarcomeric and cytoskeletal proteins, stress proteins and calcium regulators[10]. The failure of the molecular and genetic research to explain fully a complex disease like heart failure or coronary heart disease soon became evident and thus the utilization of genomics directed at the functional genomics attempted to fill the existing gaps of our understanding[11].

A review article was addressed to all 'Programs for Genomic Application' (PGA_s) that was designed for the advancement of functional genomic research in the field of cardiovascular diseases[12]. The Cardio Genomics program was assigned to study the transcriptional network of the cardiovascular system under genetic, environmental and disease stresses. This program was addressed to the transcriptional features of murine models of cardiomyopathy and human myocardium, and to gene mutations that are responsible for familial hypertrophic cardiomyopathy.

Genes and their function are unable to explain the functional complexity of a whole organ or organism as multiple proteins are produced by decipherment of one gene while many proteins are modified. Therefore it seems that the proteomics can play a significant role in explaining many cardiac cellular phenotypes. The cellular genotype is regulated by the DNA system, while the cellular phenotype is arranged by the available protein content. The proteomics technology improved the cognition of the molecular mechanisms responsible for the protein emergence and investigated the pathologies in the cardiovascular system (CVS). The proteomics technology using many experimental techniques inquired into the diseased proteome and was most informative about cardiovascular biomarkers[13-16].

The biomarkers provide more accurate and sensitive potential than clinical assessment in the identification and characterization of cardiovascular diseases[17]. Biomarkers are turned out as an important adjunct to clinical evaluation in the CVS and may be molecular in nature (hormone concentration, genome, protein expression) or reveal organ function (pulmonary or systemic artery pressure)[18,19]. While proteomics is still a new discipline, it is emerging as a technique for screening biomarkers in cells, tissues and fluids. Proteomics also identified candidate proteins altered in pathological states like atherosclerosis, dilated cardiomyopathy and ischemia/reperfusion injury.

Despite the above facts the wide clinical application of these biomarkers for preventive medicine is limited at the moment for various reasons: 1) absence of information on the prevalence and risk contribution of these markers

across population; 2) inadequate data of biomarkers inheritance and how they affect individual's risk for various diseases; 3) limited knowledge on how genetic risk factors interact with environmental factors; and 4) further interventional studies are not considered to test the effect of genetic risk factors on common diseases[4].

The use of metabolomics to genetic/chemical interventions and the way that it can be integrated with other –omics technologies demonstrated the importance of nuclear magnetic resonance (NMR) in defining metabolic profiles of intact cardiac tissues and linking them to phenotypes[20]. Grainger DJ studied the metabolic profiling generated by using NMR spectroscopy as a diagnostic test for cardiovascular diseases like identification of individuals who will suffer a myocardial infarction[21].

Recently some novel cardiac genes were identified and characterized with the help of libraries of transcript data and use of integrative methods[22-24]. The newly discovered genes are involved in mitochondrial functions, calcium metabolism and gene transcription which are expected to be useful in future cardiovascular research. In cardiac research the SB concept could be realized only if data can be gathered in different levels-genome, proteome, metabolome. More information for higher functional and interactive levels depends on advanced molecular genetics that cannot be done in man due mainly to ethical reasons[8]. This kind of genetic studies can be performed in other mammals but up today the only cardiac mammalian cell line are atria-derived HL1 cells from the adult mouse[25]. Non-mammalian model organisms recently are involved in functional molecular studies with the intention of solving complex biological problems. This is a rational experimental strategy of systemic biology that could be extended to human cardiovascular research[26].

Databases and integration

The genomic and proteomic data are voluminous but understanding their functions in a complex system like the heart is a difficult task often based on genetic manipulation and gene targeting in model organisms like the mouse or simpler organisms. This kind of simpler organisms are Escherichia coli, Caenorhabditis elegans, and Saccharomyces cerevesiae[27,28]. One of the differences of approach between the two kinds of organisms is the time of study. A single transgenic mouse takes months to develop and study while a knockout mouse takes over a year making studies very long and costly[29]. The simpler organisms are amenable to large scale mutagenesis and rapid phenotyping (genomic phenotyping) while the phenotypic characteristics can be analyzed for genome mutant libraries and a large number of alleles can be stored for further investigation[29]. The integration of pnenotypic information with genomic or proteomic data can detect novel pathways and identify new networks. In S.cerevesiae the identification of new alleles important for cellular recovery and the integration with phenotypic data on cell recovery managed to detect several protein networks significant for damage recovery[28]. Also it was discovered that some networks were associated with DNA metabolism and repair, but most of them were found to possess unexpected functions concerning the cytoskeletal remodeling and lipid metabolism.

Therefore the molecular diagnostics integrated with phenotypic characteristics give us the best chance of coming up with the right picture of SB. Probably this data integration will help in the study of cardiac biology with a holistic approach. There are several experimental data attempting to use Drosophila melanogaster for research in the field of cardiac dysfunction[30,31]. The fruit fly (Drosophila melanogaster) was the first organism with an active circulation with its genome sequenced[32]. Drosophila was proposed many times as a human disease model and several of these models were examined, particularly for neurological diseases, genetics of aging and adult cardiac dysfunction[30-33]. Human medicine and biology was enriched with many important findings emanated from studies in Drosophila or other invertebrates. Some important findings are the detection of genes regulating embryonal development in Drosophila, the identification of many components of the apoptotic machinery and the recognition of gene pathways involved in neurogenesis[34-36]. A Drosophila model suitable for studies in humans is the model with decline of the maximum heart rate with aging, a significant sign of cardiac dysfunction and a cause of restricted physical work in the elderly[37].

The heart is a complex system and the descriptive study of its molecular components and individual pathways is not adequate without integrating all the informations enclosed in different databases (Figure 1**)**. The data integration is achieved with different proteomic and metabolomic techniques[38,39]. H-NMR spectroscopy is used to combine proteomics and metabolomic analyses while the joined use of proteomic and metabolomic techniques succeeded in the integrated assessment of enzymes and their corresponding metabolites. Worldwide there are various databases that accumulate impressive amount of biological material, but the use of these databases is nearly impossible as the included material must be gathered manually[40-47]. The only method for extraction of useful biological information is the integration of the data from different sources with cross-correlation, analysis and attainment of functional networks. The larger fraction of the biological information is included in general databases while the number of the databases addressed specifically to cardiology research is restricted[48-50]. At the present time another comprehensive database is required to integrate the gathered volume of –omics material with other information such as kinetic data and mathematical models[8]. The Cardiac Integrated Database Management System (CIDMS) is considered as a database for genes expressed in the heart[51].

Cardiac networks

Previous theoretical studies have demonstrated the main aspects of evolution and deduced a simple relation-

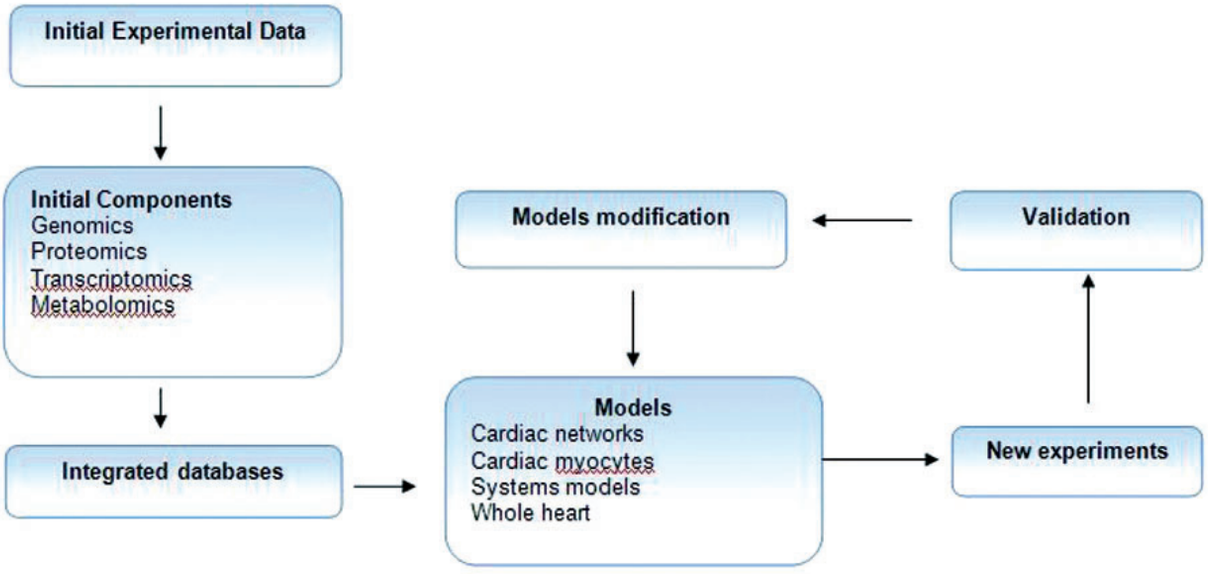

Figure 1: Systems biology schedule
Formulation of initial components from original experimental data and integration in higher-level databases. The construction of mathematical or biological models precipitates new experiments to acquire new biological parameters which are incorporated and simultaneously modify the models.

ship between genes and reproduction. This assumption neglected the important biological networks that operate during morphogenesis and development and the capacity to convert genetic potential into phenotypic actuality. Biological and mathematical methods and models have been developed to explore the interaction between networks, evolution and phenotypic variations. These methods were able to reconstruct the biochemical reaction networks of the cells and tissues. The reconstruction process of metabolic, regulatory and signaling networks are concerned with the chemical reactions or interactions that comprise these networks. All these networks are not independent but they interact between themselves and produce the complex biological phenotype[1].

Different types and properties of biological networks have been recorded and published in the literature[52,53]. Extensive biological networks were constructed and studied in simple biological systems like bacteria and yeast but the cardiovascular networks in humans and other mammals are still under development as no large-scale analysis of these networks has been undertaken. That is due probably to inadequate knowledge about interactions between metabolic networks and signaling pathways. Metabolism includes the breakdown of molecules for energy and the synthetic ability for metabolites, while signaling conducts information. A cardiovascular metabolism network has been described that presented cardiovascular metabolism as an interactive and capable network of metabolites designed for regular and predictable energy delivery[54]. Drake et al produced a network modeling integrating the CVS genetic system of the mouse with gene expression data[55]. In a recent study it was demonstrated how a network analysis of human coronary artery In-Stent Restenosis (ISR) unraveled significant components of biological pathways that could be targeted for future therapeutic intervention[56].

Cardiac myocyte models

The first endeavor for a cardiac myocyte model was the description of the cardiac action and pacemaker potentials based on the Hodgkin-Huxley equations[57-58]. This model evaluated the sodium and potassium currents during the action potential and described also the mechanistic basis of the plateau of the action potential. The more recent cardiac myocyte ionic models include twenty ionic fluxes and more than forty differential equations[29]. These models are actually functionally integrated systems models because they incorporate different separate cellular compartments such as the sarcoplasmic reticulum and the dyadic subsarcolemmal space.

Other systems models integrate functional components of cellular subsystems connected with calcium handling, energy production and myocardial filament interactions. Then more complex systems models are created with the functional integration of more than two of the above systems models. An example of this kind of complex interaction and integration is the excitation-contraction (E-C) coupling model correlating electrophysiological with energy metabolism models. Some of this kind of energy-mechanistic E-C coupling systems models have been described during the last thirty years[59].

Recently a thermokinetic model was developed explaining the cardiac mitochondrial bioenergetic behavior incorporating information and differential equations for the oxidative phosphorylation, the tricarboxylic acid cycle and mitochondrial calcium handling[60].

Cardiac systems models

The use of the classical experimental methodology to understand the biological systems often encounters difficulties particularly in complex pathophysiological circumstances. Then computational models and simulation can help to provide a quantitative interpretation of biological networks and signaling pathways. Simulation of normal and disease conditions performed on or via computer simulation ("in silico") can formulate new hypotheses and predict biological functional differences. These models include a wide range of biological systems in the level of genomics, proteomics, cells, tissues and organs. In cardiac biology and signaling networks this kind of models have been used with success in normal and diseased situations[61,62].

The mechanistic system models are low abstraction models that typically codify biochemical reactions, with kinetic rate laws as differential equations, from experimental data. They incorporate quantitative information that flows from one biological component to another and can make quantitative predictions experimentally testable[29].

These quantitative models proved efficient in explaining cardiac systems behavior which is confirmed with many such examples. Soulis et al investigated the wall shear stress oscillation in a normal human left coronary artery bifurcation computational model by applying non-Newtonian blood properties and phasic flow. They found that the lateral walls of the bifurcation, where low and oscillating wall shear stress is observed, are more susceptible to atherosclerosis[63]. Chatzizisis et al demonstrated that the geometrically correct three-dimensional reconstruction of human coronary arteries by integrating intravascular ultrasound and biplane angiography constitutes a promising imaging method for coronaries for either diagnostic or investigational purposes[64]. Hoffmann et al.predicted functional differences between IkB isoforms in computer simulation and this model tested experimentally proving the above prediction[65]. Bhalla and Iyengar demonstrated that feedback and crosstalk between neuronal signaling pathways could direct to emergent properties like bistability[66]. Luo et al used a model of an energetic metabolic network in mammalian myocardial cells and reconstructed it under normal and ischemic conditions. They indicated through simulation that the metabolic networks in myocardial cells under ischemic conditions do not maximize the production of ATP but attain a suboptimal level of energy production adapted to a moderate survival response[67].

Models of whole heart phenotype

Computational multicellular models have been useful in formally explaining the propagation of AP in the intracellular and extracellular domains. Drug induced QT prolongation has been studied in model systems designed to clarify the mechanisms of malignant arrhythmias in the genetic long Q-T syndromes ($LQTS_S$) associated with high mortality and mutations of genes encoding the fast and slow potassium channels (I_{Kr} and I_{Ks}) and the fast sodium channel(I_{Na})[68]. Anatomical models of ventricular geometry and muscle fiber construction have been built up in animals and man improving the understanding of cardiac electrical impulse propagation and wall mechanics[69-71]. The cardiac models are structurally or functionally integrated models enquiring about structural organization, voltage-gated channels, membrane transportation, force generation and SB application to study the mitochondria and the functional significance of proteomics[72-74].

A recent publication concerning the 4th Fairberg Workshop examined the various functional interactions in the cardiac system and explored the analytical approaches to the integration of the single and multiscale phenomena[75]. The goals of these Workshops are a) the interdisciplinary interaction: b) the relation of the basic phenomena to cardiac phenotype: c) the application of conceptual modeling: d) the interpretation of clinical data: and e) the enhancement of the international cooperation[75].

Conclusions

Systems biology has transformed the understanding of biology and clinical cardiology. The simple relationship between genes and disease has been replaced by biological networks and/or tissue and organ potential in an endeavor to comprehend the clinical phenotypic reality. Therefore systems biology represents a very important contemporary area of biological research in the field of cardiology.

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