

Genomics, transcriptomics, and proteomics of the ischemic heart

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Abstract

Recurring ischemia and hypertension present the major risk factors for coronary artery disease with its main acute (myocardial infarction) and chronic (chronic heart failure) manifestations. Both pathologies have a strong genetic basis. To unravel the complexity of these conditions, biomedical research is evolving from reductionism focusing on specific candidate genes toward a more integrative view ('systems biology'). Based on newly available high-throughput technologies, evidence is accumulating that genetic variability and altered gene and protein expression contribute significantly to pathophysiological outcomes. Data from "omics" studies will help to develop novel, more individualized therapeutic approaches in coronary artery disease.

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Introduction

The term "omics" is a general one describing the science of integrating the biological information about genes and proteins and finding their interrelationships, with the ultimate aims of understanding and manipulating the regulatory mechanisms (Figure 1). 'Genomics'* refers to the study of the overall structure and expression of the entire genetic inheritance, including molecular genetics (DNA level or genotype), transcriptomics (mRNA level), and proteomics* (protein level). Recently, the term "omics" has been further expanded to include metabolomics*

(study of all small-molecular-weight organic and inorganic compounds produced by the cell) [1] and kinomics (a subgroup of the proteome, encompassing all protein kinases as they exert crucial roles in eukaryotic cell biology) [2,3].

Acute and chronic ischemia

An ischemic heart is not just an "ischemic heart". Thus the following brief characterization of the most common ischemic states is given, to enable a better summary of the literature on the "omics" in ischemic

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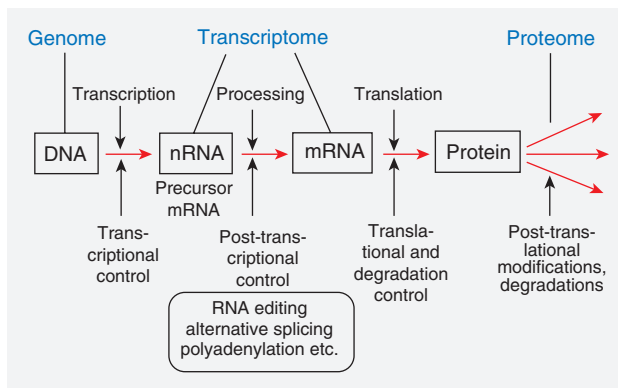


Figure 1. From genome to proteome, indicating where gene expression can be regulated or modified from transcription to posttranslation. nRNA = unprocessed nuclear RNA (precursor mRNA); mRNA = messenger RNA leaving the nucleus for joining the ribosomes; genome = the entire genetic inheritance comprised in the chromosomes; transcriptome = nRNA and mRNA together constitute the transcriptome representing the gene activity profile; proteome = the entire protein complement of a cell or a tissue.

heart disease. Myocardial infarction is the most acute manifestation of coronary heart disease (CAD). More than 60% of myocardial infarctions occur in patients older than 65 years, and age is the strongest predictor of 30-day mortality after acute myocardial infarction. Clinical and subclinical myocardial acute ischemic events may accumulate and – in the long term – generate diverse states of chronic cardiac ischemia. Protracted reduction of blood flow or repetitive episodes of ischemia may lead to the states called “preconditioning”, “stunning”, and “hibernation” [4–7]. Stunning is characterized by contractile dysfunction while blood flow and metabolism remain normal; hibernation presents a more severe state in which blood flow and energy production are reduced in combination with almost no contractility. Persistent stunning can lead to hibernation [8]. In reality these two states are gradually interchanging, and cannot be clearly distinguished. Very little or no cell death occurs during stunning and hibernation and, consequently, these states are reversible [6]. In contrast to stunning and hibernation, ischemic and pharmacological preconditioning have been reported to have a beneficial effect on long-term outcome in patients with chronic ischemic heart disease [9,10]. Clinically and subclinically acute ischemic events may accumulate over a period of time, generating a state of chronic cardiac ischemia. Unlike acute ischemic episodes, whatever the nature of a chronic ischemic state, transcriptional profiling poses difficulties for analysis and interpretation. In particular, chronic human heart disease may present widely varying transcriptome and proteome patterns, influenced by the patient’s age, sex, and inheritance, the time point in the course of the disease, and the underlying disease etiologies such as atherosclerosis, hypertension, or diabetes.

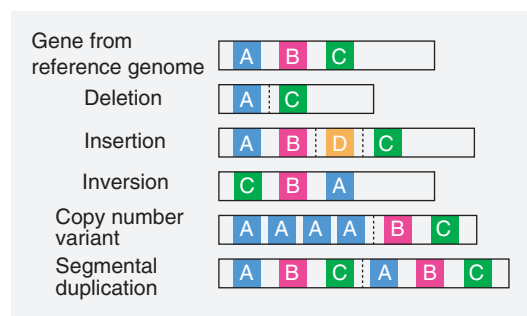


Figure 2. Common genomic variations other than single nucleotide polymorphisms. (Adapted from Check [11], with permission.)

In addition, valid controls for comparison are difficult to obtain in human studies. For these reasons, the available information relates mostly to animal models.

Genetics of ischemic heart disease

A genome-wide search using high-throughput sequencing technologies is required to map single nucleotide polymorphisms (SNPs) and the different structural variants of DNA such as microsatellites, minisatellites, insertions or deletions, reversions, multiple gene copy numbers, and expanded nucleotide repeats (Figure 2) [11,12]. The most comprehensive catalog of known structural variations is the Database of Genomic Variants (<http://projects.tcag.ca/variation/>) with around 4000 entries at ~2200 loci, covering a staggering 405 Mb (14%) of the human genome sequence. The sizes of the entries in the Database range from 1 kb to 3.89 Mb, with a median of 103 kb. Alternatively, as of March 2008, the International HapMap Consortium (<http://www.hapmap.org>) has developed a map covering the entire human genome, representing ~3.9 million SNPs or one SNP per ~700 bp [13,14]. The HapMap project is designed to provide the means to link genetic variants to the risk for specific diseases and to correlate SNP profiles with drug response patterns. Because of structural variants and SNPs, the identity of the genomic sequence between two individuals is reduced to a mere 85%.

In CAD and its associated risk factors, several genes predispose to the final phenotype, each contributing only a modest amount. Thus only after all genetic modifiers and their functions have been identified will it be possible to develop more appropriate therapies. Furthermore, multigene disorders require genome-wide association studies involving genotyping hundreds of thousands of DNA markers in a large number of individuals, and with replication in independent populations. The first confirmed locus associated with CAD is located on chromosome 9p21.3 [15–18]. In essential hypertension – the most common risk factor for cardiovascular morbidity and

mortality – genome-wide linkage analyses provide some consistency of linkage results in few chromosomal regions on chromosomes 1, 2, 3, 17 and 18 [19,20].

Transcriptome after myocardial infarction

The changes in the transcriptional profile found after acute global ischemia followed by reperfusion or in vivo after regional ischemia strongly depend on the duration or the severity, or both, of the ischemic insult [21–24]. A microarray analysis of the rat heart during infarction and remodeling revealed a strong increase in the expression of atrial natriuretic peptide and smaller but significant changes in genes involved in protein synthesis, cytoskeletal and extracellular matrix proteins, and genes related to energy metabolism [24]. The infarct zone (50% of the left ventricle) associated with a permanently ligated left anterior descending coronary artery was found to be characterized within the first 24 h by transforming growth factor β -1 and an overriding depression in transcription, signal transduction, inflammation, and extracellular pathways. Within the same first 24 h of infarction, in the unlesioned remote zone, expression of genes was reciprocally activated, including interleukins 6 and 18 among others, and tumor necrosis factor- α [21]. At day 28 after the ligation procedure, genes for signal transduction, inflammation, transcription factors, metabolism, and detoxification – all classes previously depressed in the day-1 infarct zone – dominated the day 28 infarct zone. Genes for extracellular matrix components were also high, whereas indices of cell growth and replacement remained low. Gene expression in the remote zone on day 28 followed the day-1 pattern, with a reduction in the number of affected genes. Thus survival in the face of a massive infarct induces a compensatory strategy in the remote zone, followed by a delayed activation of the same pattern in the infarct zone. The transcriptional program activated in the surviving myocardium after a long period of ischemia thus supports the concept that postinfarct remodeling proceeds globally as a result of the sustained pathologic activation of initially compensatory molecular responses [21,25].

Transcriptome in chronic ischemia and human heart failure

Ischemic heart disease is the most common underlying cause eventually leading to left ventricular hypertrophy and heart failure. Non ischemic hypertrophic (HCM) and dilated (DCM) cardiomyopathies (25–35%) may derive from hypertension (~17%), valve pathologies (~13%), and hereditary gene defects in the contractile and cytoskeletal proteins

(>20%). The concept that genes encoding proteins with similar functions or involved in the same pathway are responsible for a particular disease has led to the hypothesis of a “final common pathway” operating in DCM [26]. The hypothesis may be further supported by the fact that consistent changes in genes related to energy metabolism (already detected early in acute ischemia, as reported in the previous section) occur across HCM, DCM, and ischemic (ICM) cardiomyopathies, despite the conditions having quite different etiologies [27]. Moreover, in contrast to previously reported data [28], it has been found that ICM and non ischemic cardiomyopathy exhibit substantial heterogeneity at the transcriptomic level and do not display a characteristic etiology-specific signature [29]. If the concept of the “final common pathway” postulated for DCM with different etiologies is extrapolated to endstage heart failure, one would expect that a common signature of the transcriptome may develop in the terminally failing heart, independent of the initial disease. Because this important question could not be solved by a series of studies examining sets of genes in the human cardiac transcriptome associated with heart failure [30], a novel approach was adopted in which it was hypothesized that a discrete set of cardiac transcription factors may regulate gene activity in the pathogenesis of heart failure. Insight into transcription factor function was obtained by comparing data on microarray gene expression in cardiac tissue collected at a single time point from patients with advanced heart failure (DCM or ICM) against murine genome sequence data. The results indicated that, besides the known transcription factor families, nuclear factors of activated T-cells (NFAT), myocyte enhancer factor-2 (MEF2), Nkx, and GATA, several additional transcription factors are active in human heart failure, notably the Forkhead Box (FOX) family (FOXC1, C2, P1, P4, and O1A). However, NFAT activity is more closely associated with heart failure in DCM (suggesting a more prominent role for abnormal Ca^{2+} signaling and calcineurin-mediated transcription), and the transcription factor family CCAAT-enhancer-binding proteins (C/EBP) (which are modulated by inflammation and mitogen-activated protein kinases) with heart failure in ICM. These findings would indicate that different subsets of genes are altered in different types of human heart failure, possibly reflecting the different disease etiologies [30].

Studies of patients with a left ventricular assist device (LVAD) ([31] and references therein) have shown that the failing human heart retains plasticity. LVAD support induces a regression of pathological hypertrophy, improvements in contractile performance and contractile reserve, regression of pathological electrophysiological markers, and reduction

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in myocardial cytokines and apoptosis. However, it is sobering to note that, from more than 3000 genes showing significant changes in failing hearts, only 16 reverted to normal levels (four displaying overcorrection) and 27 showed only partial reversion after LVAD treatment. Almost 200 genes exhibited persistence or even exacerbation of the dysregulation associated with heart failure [31]. This indicates transcriptional “hysteresis”, in as much as many transcriptional changes in severe heart failure do not follow the functional recovery achieved by the LVAD treatment. More generally, it seems

that the molecular adaptations leading to myocardial recovery may not simply be the inverse of those associated with the development of heart failure.

In a recent clinical study of our own, in which we attempted to move in the direction of functional genomics, towards which the future is aiming [32], we demonstrated correlations between the transcriptional changes after cardiac surgery (with and without cardioprotective treatment: sevoflurane compared with propofol) and cardiac function and changes in biomarkers (Figure 3).

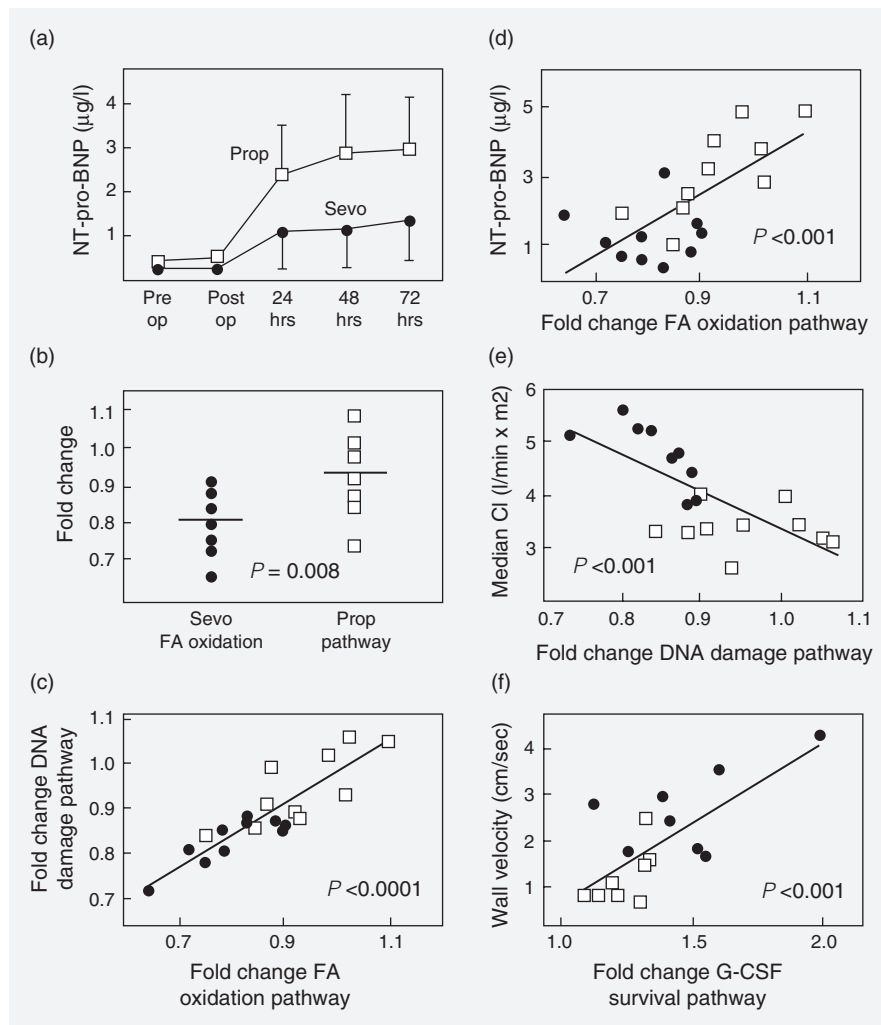


Figure 3. Postoperative blood levels of N-terminal pro brain natriuretic peptide (NT-proBNP) and correlations between anesthetic-induced transcriptional phenotypes with postoperative cardiac function recovery of patients subjected to coronary artery bypass graft (CABG) surgery. (A) Plasma levels of NT-proBNP are significantly higher in the PROP (squares) than the SEVO (circles) group from 24 up to 72 h after operation (time effect $P < 0.001$; treatment effect $P < 0.002$). (B) Differential regulation of the FA oxidation pathway. (C) Correlation between FA oxidation and DNA damage pathway. (D) Correlation between FA oxidation and NT-proBNP. (E) Correlation between DNA damage pathway and cardiac index (CI). (F) Correlation between diastolic wall motion velocity, as determined by tissue Doppler flow transesophageal echocardiography, and G-CSF (granulocyte-colony stimulating factor). Collectively, these data imply that higher FA oxidation, as observed with propofol, put the heart at higher risk of postoperative contractile dysfunction. Reduced DNA-damage signalling may reflect the protection resulting from metabolic fuel shift. G-CSF survival pathway may have a direct role in cardiac fuel selection by regulating STAT3-mediated insulin sensitivity in the heart. Members of this pathway are JAK2/3, STAT3, vascular endothelial growth factor, and protein kinase B, which promote cell survival and angiogenesis. SEVO = sevoflurane; PROP = propofol. (Modified from Lucchinetti et al [32], with permission.)

Proteomic analysis in heart disease

Efforts are continuing to characterize the protein-related changes associated with cardiac dysfunction after ischemia-reperfusion injury. Changes in the abundance of proteins and, in particular, posttranslational modifications, increase with increasing severity and duration of experimentally applied ischemia-reperfusion injury [33–35]. Some of these changes are also present in chronic ischemic hearts. In a dog model of pacing-induced heart failure, Heinke et al [36] found a decreased abundance of the enzymes of mitochondrial oxidative phosphorylation, an increased expression of glycolytic enzymes, and varying changes in structural and cytoskeletal proteins. Proteomic profiles of human DCM and patients with ischemic heart disease [37,38] have revealed that a large proportion of the protein changes are associated with mitochondria and energy metabolism, including pyruvate dehydrogenase and isocitrate dehydrogenase subunits, creatine kinase M, and fatty acid binding protein. For this reason, the field of investigation has now moved on to cellular fractionation combined with proteomic analyses, to achieve better tracking of subcellular protein translocation and posttranslational modifications. Some recent studies focused on phosphorylation of mitochondrial proteins in relation to their function. Schwertz and coworkers [39] subjected rabbit hearts to 60 min of ligation of the left anterior descending coronary artery followed by 3 h of reperfusion and found a 4-fold increase in phosphorylation of voltage-dependent anion channel-1 compared with that in control hearts. Our group reported a novel phosphorylation site in adenine nucleotide translocase 1 (at residue Tyr194) [40] that might be involved in coordination of mitochondrial energy metabolism and cardioprotection. In a genetically modified yeast model, this phosphorylation site was critically linked to cellular respiration. Tyr194 phosphorylation was equal in mitochondria from control and protected – that is, pre- and postconditioned – hearts, whereas ischemia-reperfusion damage alone significantly reduced Tyr194 phosphorylation [40].

Outlook

Future developments in the “omics” field will further modify the definition of complex cardiovascular disease states. To date, the initial promise that “omics” may support the identification of disease biomarkers and potential targets for drugs, and thus improve therapy, has been partly fulfilled. Importantly, identified biomarkers need to be carefully validated in large populations of patients, particularly when they are to be used to predict long-term prognosis [41,42].

*See glossary for definition of these terms. ■

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