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Review

Genetic and non-genetic determinants of clinical phenotypes in cardiomyopathy

Seitaro Nomura

Department of Cardiovascular Medicine, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

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ABSTRACT

Cardiomyopathy, a leading cause of death worldwide, is etiologically and phenotypically heterogeneous and is caused by a combination of genetic and non-genetic factors. Major genomic determinants of dilated cardiomyopathy (DCM) are titin truncating mutations and lamin A/C mutations. Patients with these two genotypes show critically different phenotypes, including penetrance, coexistence with a conduction system abnormality, cardiac prognosis, and treatment response. The transcriptomic and epigenomic characteristics of DCM include activation of the DNA damage response, metabolic reprogramming, and dedifferentiation. The proteomic and metabolomic signatures of the DCM heart include a rigorous dependency for free fatty acids, activation of the stress response, and metabolic reprogramming. Proteomic and metabolomic analyses of blood show a distinct immune response and an unexpected link with pathology-specific microbiota in DCM. The direct integration of multi-omics data will not only elucidate inter-omics associations but also enable omics-based patient stratification, which will lead to a deeper understanding of cardiomyopathy and the development of precision medicine in cardiology.

Contents

Introduction

Cardiomyopathy is caused by a combination of genetic and non-genetic factors. Since the discovery of a mutation in the myosin heavy chain 7 gene in patients with hypertrophic cardiomyopathy (HCM) [1] and mutations in the actin, alpha, cardiac muscle 1 gene in patients with dilated cardiomyopathy (DCM) [2], more than 50 genes have been identified as causal for the development of cardiomyopathy [3]. In particular, DCM is a leading cause of heart failure, and patients often need left ventricular assist device implantation and/or heart transplantation [3]. Recent studies with comprehensive genomic analysis have revealed some notable genotype–phenotype associations in DCM [3], suggesting that the established genomic factors are helpful for predicting the phenotypes of patients. In addition, epigenomic, transcriptomic,

E-mail address: senomura-cib@umin.ac.jp.

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proteomic, and metabolomic analyses have uncovered non-genomic factors associated with the clinical phenotypes of DCM. Historically, the terminology of cardiomyopathy has been based on morphological characteristics, but these multi-omics studies are now reshaping the pathological structure of cardiomyopathy. Here, I provide a concise review of recent advances in multi-omics analyses for identifying the genetic and non-genetic determinants of clinical phenotypes in cardiomyopathy, and present the future perspectives of this rapidly developing field.

Genomic architecture of cardiomyopathy

Target enrichment system and next-generation sequencing technologies have enabled simple and efficient comprehensive gene screening for cardiomyopathy. Recent comprehensive targeted sequencing studies showed that approximately 40% of patients with DCM have pathogenic mutations in genes reported to be causal for cardiomyopathy [4,5]. Although there are some ethnic differences in the proportion of mutated genes, titin (*TTN*) truncating mutations and lamin A/C (*LMNA*) mutations are considered to be major factors for the development of DCM.

TTN truncating mutations are the most frequent cause of DCM [6] and account for 15–25% of DCM cases [4,5]. The clinical phenotypes of *TTN* truncating mutations include low penetrance, non-coexistence with a conduction system abnormality, relatively good prognosis, sex differences in prognosis (males have a worse prognosis than females), and a tendency for left ventricular reverse remodeling [3–5]. In spite of the low penetrance of *TTN* truncating mutations, additional cardiac insults such as pregnancy and alcohol abuse might cause cardiac dysfunction [7,8]. Although cardiomyopathy patients with *TTN* truncating variants (TTNtv) show severe left ventricular dysfunction at diagnosis, they tend to present with a good response to appropriate medical therapy and their cardiac function improves drastically [5]. A ribosomal profiling assay showed TTNtv-position-independent nonsense-mediated degradation of the mutant allele and a signature of perturbed cardiac metabolism [9].

LMNA mutations are the second most frequent cause of DCM [10] and account for 5–10% of DCM cases [4,5]. The clinical phenotypes of *LMNA* mutations include high penetrance, young onset, coexistence with a conduction system abnormality, poor prognosis, poor response to medical therapy, and frequent need for heart transplantation [3–5,11]. Due to the frequent coexistence of *LMNA* mutations with a conduction system abnormality, The European Society of Cardiology guidelines recommend the use of an implantable cardioverter defibrillator in patients with DCM and a confirmed disease-causing *LMNA* mutation in order to prevent sudden cardiac death [12]. *LMNA* mutations are considered to be linked to a defect in mechanotransduction [13] and the development of laminopathy, which shows various clinical symptoms including skeletal and/or cardiac muscular dystrophy, lipodystrophy, dysplasia, neuropathy, leukodystrophy, and premature aging. Therefore, studies investigating the underlying mechanisms by which each variant causes each phenotype are necessary to dissect *LMNA*-associated cardiomyopathy.

At the present time, panel-based genetic screening is unable to identify the causal variants in almost 60% of patients with familial DCM [4,5], which suggests that unknown genomic factors might be responsible for the development of DCM. Recent studies with whole-exome sequencing have identified myosin light chain kinase 3 and DNA topoisomerase III alpha as novel causal genes for DCM [14,15]. Whole-genome sequencing of patients with HCM highlighted the importance of intronic variants causing splicing abnormalities [16]. The identification of these remaining genomic factors will lead to an improvement in our ability to give a correct diagnosis. On the other hand, comprehensive genetic testing sometimes identifies multiple causal variants in one patient with cardiomyopathy. Whole-exome sequencing of a family with cardiomyopathy suggested that a subset of HCM might be oligogenic in nature, that is, caused by multiple pathogenic variants that do not co-segregate perfectly with the phenotype [14,17], which indicates that the study of variant-phenotype associations is necessary to elucidate the extent to which each variant contributes to each cardiac phenotype.

Transcriptomic architecture of cardiomyopathy and heart failure

As individual cells constitute the basic units of gene regulation, the phenotype and function of each cell are considered to be determined based on its transcriptional programs. A large-scale cardiac transcriptome analysis of patients with DCM revealed that RNA transcription, splicing, and allele-specific expression are important determinants of the DCM phenotype and are controlled by genetic factors [18]. Most of the differentially-expressed genes (DEGs) have been reported to be involved in the development of DCM, and most of the identified differences in alternative splicing and allele-specific expression have been implicated in the etiology of DCM [18]. Transcriptome analysis also revealed that incomplete differentiation is a molecular characteristic of pediatric DCM [19].

Recently developed single-cell transcriptome analysis enables us to identify differences in molecular profiles at the single-cell level, to extract the co-regulated gene networks, and to reconstruct the single-cell trajectory during development and disease. Single-cardiomyocyte RNA-sequencing (RNA-Seq) of heart failure model mice reconstructed a trajectory during cardiomyocyte remodeling at the single-cell level, and distinguished the molecular profiles of the adaptive phenotype and failing phenotype in the failing heart [20]. By integrating single-cardiomyocyte morphology and transcriptome with the epigenome, the authors showed that mitochondrial gene expression was correlated with cellular hypertrophy and was linked with extracellular signal-regulated kinase 1/2 and nuclear respiratory factor 1/2 signaling transcriptional networks. They also found that DNA damage-induced p53 signaling was activated at the induction of failing cardiomyocytes, and they generated cardiomyocyte-specific p53 knockout mice to show that p53 activation was necessary for the induction of failing cardiomyocytes, which are characteristic for repressed mitochondrial gene expression and morphological elongation.

Single-cell RNA-Seq also revealed that cardiomyocytes from patients with DCM have significant transcriptional heterogeneity and that this heterogeneity can be explained by the expression of two gene modules, which include DNA damage response genes and mitochondrial genes [20]. The authors further showed that the expression levels of these two gene modules can classify the pathogenesis of each patient and can predict their cardiac prognosis after left ventricular assist device implantation [20]. These findings highlight the importance of the use of cardiomyocyte transcriptomes for assessing and predicting cardiac phenotypes.

Epigenomic architecture of cardiomyopathy

Epigenomic information provides a crucial regulatory layer between genomic information, environmental factors, and transcriptomic information. DNA methylation profiling of the heart identified several loci showing DCM-specific methylation patterns, where methylation levels were correlated with the transcription levels of their target mRNAs [21]. For example, the DNA methylation levels of the heart and neural crest derivatives expressed 2 (*HAND2*) locus are associated with the mRNA expression levels of not only *HAND2* but also its downstream genes. The authors also conducted DNA methylation profiling of

blood samples from the same patients and revealed that DNA hypomethylation at the natriuretic peptide A (*NPPA*) and *NPPB* loci in the heart was conserved even in the blood.

However, since epigenomes are critically different between cell types, cell-specific epigenomic information is necessary to identify the cell types that are affected in pathological conditions. Actually, recent studies with cardiomyocyte-specific epigenomic profiling, such as histone modifications and DNA methylation, revealed that pathological gene expression in heart failure is accompanied by changes in active histone modifications without major alterations in DNA methylation or repressive chromatin modifications [22]. The authors also showed that the cis-regulatory elements of cardiomyocytes were enriched for cardiovascular disease-associated variants. Integrative analysis of epigenomic profiling and phenotypic information would elucidate epigenomic characteristics that can be used to assess and predict cardiac phenotypes. Recently evolved single-cell technology would be useful for simultaneously assessing cell type-specific epigenomic profiling in the heart [23].

Proteomics architecture of cardiomyopathy

Proteins translated from mRNAs mainly function in cells. Proteomic analysis of the heart of patients with DCM not only revealed a significant overlap between DEGs and differentially expressed proteins (DEPs), but also showed that these DEGs and DEPs were related to energy metabolism, the maintenance of cell structural integrity, and the stress response [24]. Additional measurements of protein turnover may allow the discovery of post-transcriptional regulatory mechanisms and the identification of essential proteins not found from steady-state transcript and protein abundance data [25]. Turnover of proteins involved in membrane remodeling and glycolysis was positively correlated with cardiac hypertrophy, whereas that of proteins involved in fatty acid oxidation was negatively correlated with hypertrophy. A recently reported region and cell type resolved quantitative proteomic map of the human heart may be useful for dissecting the pathogenesis of DCM [26]. Chemical cross-linking mass spectrometry has provided structural information on key protein systems in the heart including the sarcomere and oxidative phosphorylation complexes [27].

One-third of all cardiac proteins are known or predicted to be phosphorylated to function. Phosphoproteome profiling of the heart from DCM model mice with a mutation in the phospholamban gene identified a change in the phosphorylation of Notch signaling components [28]. Furthermore, phosphoproteome analysis of the heart from non-ischemic heart failure model guinea pigs showed that mitochondrial reactive oxygen species drive MAPK target phosphorylation during end-stage heart failure [29].

Serum proteomic profiling in DCM model dogs showed that up-regulated proteins were associated with G protein-coupled receptor signal transduction, complement cascade activation, lipoprotein particle dynamics, elastic fiber formation, and the respiratory electron transport chain [30]. Proteomic analysis of circulating extracellular vesicles in patients with DCM revealed that the up-regulated proteins were involved in the stress response, platelet degradation, blood coagulation, and the complement pathway [31]. Recent integrative analysis of genomic, blood proteomic, and microbiomic data from the same individuals showed that the genome contributed the most to the concentrations of immune-related proteins, whereas the gut microbiome contributed the most to proteins involved in metabolism and intestinal health [32]. Another genome-wide mapping study of plasma protein quantitative trait loci identified putative causal genes and pathways for coronary artery disease [33].

Metabolomic architecture of cardiomyopathy

Metabolomic profiling of the heart from *Lmna* knockout DCM model mice showed increased concentrations of proline and methylhistidine residues, suggesting increased myofibrillar and collagen degradation, as well as a decrease in several citric acid cycle intermediates and carnitine derivatives, indicating reduced energy metabolism [34]. A previous study showed that an acute reduction of free fatty acid uptake led to an abrupt decline in cardiac function in patients with DCM, implying that reducing the supply of free fatty acids to a metabolically stressed heart might have a negative effect on cardiac efficiency [35]. Plasma metabolic analysis revealed that the plasma levels of steroid metabolites, glutamine, threonine, and histidine were reduced, whereas the levels of citric acid cycle intermediates and lipid β-oxidation products were increased in patients with DCM [36]. Integrative analysis of a genome-wide association study and blood metabolomic profiling identified several significant causal links for the development of coronary artery disease [37]. Further integrative analysis of microbiome and serum metabolomics profiling identified unexpected links between intestinal microbiota alterations, circulating amino acids, and obesity [38]. Fecal metabolome profiling largely reflects gut microbial composition, and is also a novel tool to explore links among microbiome composition, host phenotypes, and heritable complex traits [39].

Data integration for future systems in cardiomyopathy

In this review, I have summarized not only the recent findings of multi-omics analyses in cardiomyopathy, but also the promising technologies and analytical pipelines for a deep understanding of its molecular pathogenesis (Fig. 1). Although most studies have

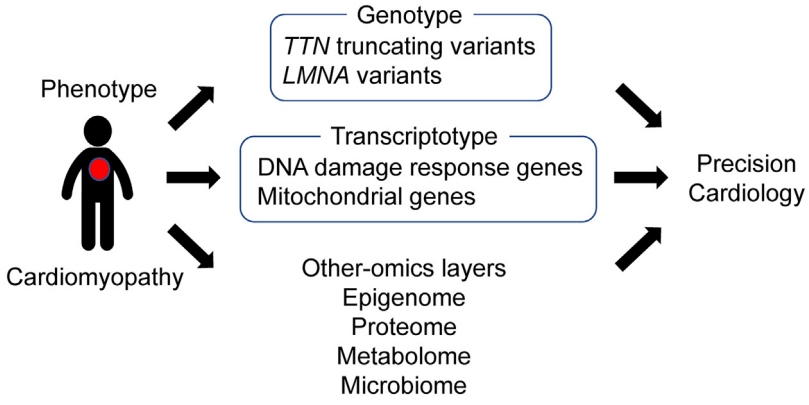

Fig. 1. Multi-omics integration of cardiomyopathy for precision cardiology.

examined the omics information associated with the development of cardiomyopathy, future studies are needed to elucidate the omics information associated with the phenotype of each patient, which includes clinical prognosis, imaging and physiological findings, and treatment response. It is also important to integrate directly multi-omics data obtained from the same patients with cardiomyopathy [40,41]. This direct integration will not only elucidate inter-omics associations but will also enable omics-based patient stratification, which will be applied for the development of novel tools or pipelines for use in the rapid, simple classification of cardiomyopathy. Omics-based stratification will lead not only to the appropriate treatment choice but also to the identification of difficult-to-treat pathology, which will be clarified by future studies, for example, using patient-derived induced pluripotent stem cells and/or knock-in animals with pathology-specific mutations.

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Disclosures

The author declares no conflict of interest.

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