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Editorial

Quaero muneris: Exploring microRNA function in cardiovascular disease☆

In antiquity, the Roman Empire was known for its technological achievements and innovations that delivered it a contemporary, strategic advantage over neighboring civilizations and played an important role in shaping sequential civilizations, including ours. The Romans were masters in adaptive innovation, revising existing concepts and inventions by earlier empires, most notably the Etruscans, Greeks and Egyptians, by taking them to new and innovative levels of use and functionality. Several Roman technological feats in different areas like civil engineering, construction materials, transport technology, and military technology were surprising achievements until the 19th century, and some, such as the invention of the architectural arch, have remained untouched to this day. A central concept in modern empirical science and Physiology, the science of the function of living organisms, is that all evidence must be empirical, or empirically based. We likely owe our empirical scientific methods, where understanding the ultimate functionality of new findings are continuously reiterated and scrutinized, to a great part to ancient Roman philosophy, where a keen eye for usefulness and functionality of new technology was central in everyday life.

1. microRNA control of cardiomyocyte hypertrophy

MicroRNAs (miRs) have rapidly gained attention in the field as seemingly crucial, novel regulators of cellular morphology and function. MicroRNAs are ~22 nucleotide long, evolutionary conserved, non-coding RNA molecules encoded by the genome and designed to play a role in post-transcriptional gene regulation [1,2]. MicroRNAs exert this regulatory function by imperfect Watson–Crick base pairing to the 3′ UTR region of protein coding messenger RNAs (mRNAs), where they subsequently suppress translation and/or induce mRNA degradation. MicroRNAs were initially thought to act as subtle 'fine tuners' of gene expression [3], but increasing evidence suggests that the regulatory functions of microRNAs are far more crucial for the cell, especially under stressful conditions.

The finding that microRNA genes are centrally positioned in cellular survival and proliferation in various forms of cancer, and inhibition of single microRNAs is virtually devoid of toxicity and promises to become the next generation therapeutics, sparked interest in this class of non-coding RNAs (ncRNAs) in the cardiovascular system. Recent studies have started to unveil unexpectedly powerful roles for single microRNAs in a variety of cardiovascular diseases including pathological left ventricular remodeling, the primary response of the heart to stress and leading cause of heart failure [4]. Given the clinical prognostic value of hypertrophy, much effort is centered on the identification of genes and pathways driving cardiac remodeling for future rational drug design in heart failure therapy [5,6]. We have witnessed the first screens for differentially regulated microRNAs in left ventricular hypertrophy [7,8], the first manipulations to impact microRNA maturation in the postnatal cardiac context [9], followed by the far more time-consuming studies that focus on single microRNA genes, discovery of microRNA target genes and meticulous description of their mechanistic role in overall cellular function [10–14]. These combined efforts have yielded valuable insight of individual microRNAs that have the ability to regulate cardiac remodeling through their specific effects on signaling cascades (miR-133, miR-1, miR-9, miR-98, miR-199b, miR-23a), sarcomere organization (miR-1, miR-133, miR-208a/b and miR-499) and inter-cellular communication (miR-29 and miR-21), to name a few (for a recent review [2]).

With the rapidly expanding inventory of information on the workings of our genome in the post-genomic era, laborious one by one tests purely based upon array based data of differentially expressed genes, required a high-throughput system to screen simultaneously for genetic effects individual genomic units (genes, ncRNAs) and cellular function, also referred to as functional genomics. The usefulness and advantages of such a functional screening approach were displayed by the use of libraries with viral vectors expressing short hairpin RNA for several thousand genes, although these short hairpin RNAs are only targeted to knockdown protein-coding genes [15]. Voorhoeve et al. [16] constructed a genetic screening assay of microRNAs using a retrovirus vector library expressing miRNA minigenes and a barcode microarray. These phenotype-based approaches, previously developed in other biomedical fields, enabled identification of essential genes critical in proliferation in cell lines that do not necessarily have mutated sequences or aberrant copy numbers, a feat that resembles the cardiomyocyte hypertrophy response. In this issue of the Journal of Molecular and Cellular Cardiology, Jentszch and colleagues have now established a high-throughput, cell-based, one-well/one-gene functional screening platform. This was accomplished by combining a synthetic library of easily transfectable, chemically synthesized, precursor sequences of 230 individual microRNAs with an automated edge detection setup to identify microRNA pathways that govern myocyte hypertrophy [17].

The daunting task to screen 230 different RNA molecules in parallel for their effects on cell size required the scientists to develop a format that uses appropriate cells with high purity and a reliable readout parameter. The most widely used cellular system for cardiomyocyte hypertrophy still employs primary cells isolated from neonatal rat hearts [18]. The authors have optimized the existing isolation protocols and plating density allowing for high cell purity, while employing fluorescence techniques to discriminate cardiomyocytes from other

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cell types. The combinatorial input yielded the first in its kind of fluorescence-based microscopy in an automated setup with data acquisition by automated microscopy at a 96-well setup. Applying this strategy, up to 2700 cells could be analyzed per well using an image segmentation algorithm, allowing for the development of a unique technology to culture and transfect a large collection of microRNAs in a 96-well format, coupled to a robust edge detection system capable of screening 230 microRNAs individually and in parallel for their effect on cardiomyocyte size. The automated setup with edge detection will also be an invaluable resource to screen for small molecules with pro- or anti-hypertrophic properties on an industrial level, viral libraries harboring shRNA targeting protein coded genes, or ncRNAs, to mention a few.

Screening the aforementioned microRNA library yielded microRNAs previously known to induce a pro-hypertrophic response, providing an internal control for the validity of the functional screen. More excitingly, several microRNAs previously not recognized as pro- or anti-hypertrophic were also detected, including the pro-hypertrophic potential of miR-212, miR-365, miR-22, miR-30c, miR-30d and the anti-hypertrophic potential of miR-27a, miR-27b and miR-133a. An additional fundamental breakthrough pertained to the quantitative analysis of the expression level of prohypertrophic microRNAs in primary cardiomyocytes, which indicated a rather low level of correlation of the phenotypic effects of individual microRNAs and their expression level. As reiterated above, the majority of the one microRNA–one assay experimental approaches performed to date have focused on microRNAs whose cellular levels change under disease causing conditions, based on the theoretical assumption that disease relevance and deregulation are tightly linked (reviewed by Small et al. [2]). A particularly strong feature of this study is the observation that this hypothesis is clearly incorrect, suggesting that the inventory of cardiovascular functional microRNAs is larger than previously hypothesized.

Next to the advantages of this new technological development, there are also some points of improvement, such as the use of neonatal versus adult cardiomyocytes, which may react differently during hypertrophic remodeling due to the different developmental stages in which the cells reside. It is feasible to assume that some of the microRNAs in this library will display a different expression level in the neonatal versus the adult myocyte [7], although multi-day, long term cell culture of adult myocytes without the inevitable dedifferentiating and degenerative processes associated with latter culture procedures remains a serious obstacle that precludes high-throughput functional genomics screens. A second cautionary remark pertains the current use of a precursor microRNA library, designed to achieve single microRNA overexpression. In theory, it is possible that certain microRNAs, normally absent in the heart, are overexpressed at (supra)physiological levels and produce a measurable morphological alteration in cardiomyocytes, which would be scored as false positive hits in the screen, but which may have little to do with cardiovascular (patho)physiology. To avoid this caveat, more recently developed higher content microRNA knockdown libraries with near genome-wide microRNA coverage would avoid latter pitfall. Notwithstanding these critical appraisals, this study illustrates the power of RNAi-based functional genomics to identify novel genes, pathways, and pharmacologic agents that impact a biological phenotype and operate outside of preconceived mechanistic relationships. In keeping with our Roman ancestry, this new technological feature will aid in tackling contemporary genetic complexity and realize our Physiological quest to search for functionality, ultimately providing us with a strategic advantage over adjacent fields of research.

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Disclosures

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