Microarray-based genetics of cardiac malformations

Jan Miertus*, Antonio Amoroso*§

*Division of Genetics, Department of Reproductive and Developmental Sciences, University of Trieste,
§Genetics Service, IRCCS Burlo Garofolo, Trieste, Italy

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One of the most revolutionary approaches in human genomics is DNA microarray technology. Latest developments have brought this technology to a widespread use. In this paper we discuss its usefulness especially for the study of the genetic component in congenital heart disease as a model of multifactorial disease and the possible clinical applications in the near future. Malformations of the heart and blood vessels account for the largest number of human birth defects. The susceptibility of the heart to developmental anomalies reflects the complexity of the morphogenetic events responsible for the heart formation. The genetics of congenital heart disease points to the existence of powerful disease modifiers. Tissue analysis of gene expression with cDNA microarrays provides a measure of transcriptional or posttranscriptional regulation. Large-scale partial sequencing of cDNA libraries generating expressed sequence tags is an effective means of discovering novel genes and characterizing transcription patterns in different organs and tissues. The qualitative and quantitative analysis of genes expressed in cardiac tissue by means of comparison of expression patterns related to the normal and to the pathological tissue may be of great importance for the study of cardiac pathologies. The variation in phenotypic penetrance and severity suggests that if we can identify high-risk individuals, a reduction in infant morbidity might be possible by altering environmental or maternal factors.

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Address:

Prof. Antonio Amoroso

*Servizio di Genetica
IRCCS Burlo Garofolo
Via dell'Istria, 65/1
34137 Trieste
E-mail:
amoroso@burlo.trieste.it*

Recent years have witnessed technological advances that have been changing the paradigms of population screening. At the forefront of the revolution in human genomics is DNA microarray technology that evaluates expression levels or genotypes of thousands of genes simultaneously by means of miniaturization and parallel processing. Traditionally, DNA microarray technology has been the province of research and high-end clinical diagnostics. However, the latest developments and new manufacturing procedures have made its use widespread mostly because of the decrease in costs that made it a feasible and affordable tool. It also consolidates existing laboratory tests into a single, more efficient and economical format enabling us to perform thousands of assays in a relatively short time[1]. Clearly, the field of molecular biology and human genetics is in transition. New technologies are necessary to explore uncharted waters. This is valid for example for the early detection of cardiac malformations.

Malformations of the heart and blood vessels account for the largest number of human birth defects, with a reported incidence of about 1% of live births, and among stillbirths the frequency has been estimated to be 10 times higher[2]. Heart formation requires complex interactions among cells originating from multiple embryonic lines. Formation of the heart requires migration, differentiation, and precise interactions among multiple embryonic cell types. The susceptibility of the heart to developmental anomalies reflects the complexity of these morphogenetic events. Recent studies have begun to reveal the genetic pathways that control cardiac morphogenesis. However, our understanding of the molecular circuits that drive the onset of complex cardiac diseases still remains primitive. The shortness of informative human mutations, the high frequency of embryonic lethality in gene-targeted mouse models and the complexity of physiological endpoints have made it difficult to uncover new disease pathways. On the other hand, the recent identification of the genes involved in the early phases of heart development has provided an impetus for studying their functions and for the improved understanding of cardiovascular diseases and forms the basis for future therapeutic interventions[3]. The challenge is to identify modifiers of the disease, and to design new ther-

apeutic strategies to interrupt the underlying disease pathways[4].

It is clear that congenital heart defects represent a heterogeneous group of pathologies. Congenital heart diseases are usually classified according to their anatomical arrangements, but it may be more useful to consider them according to their embryological origins (Table I).

Congenital heart defects constitute the largest group of anatomic congenital defects in humans and are the principal cause of mortality in childhood. Traditionally, most cases are considered as the result of complex interactions between genetic susceptibility and environmental stress (multifactorial cause). The susceptibility to multifactorial diseases depends on slight modifications (such as single nucleotide polymorphisms) of more than one gene rather than on mutations in a single gene. Interestingly, up to 10% of congenital heart defects are caused by chromosomal anomalies. Single mutant genes may produce a number of common, nonsyndromic congenital heart lesions. Estimations suggest that single genes cause more than 5% of congenital heart defects[2].

The genetics of congenital heart defects points to the existence of powerful disease modifiers. A wide phenotypic spectrum is seen in patients harboring identical disease alleles. The variation in phenotypic penetrance and severity suggests that if we can identify high-risk individuals, a reduction in infant morbidity might be possible by altering the environmental or maternal factors. This risk reduction strategy, for example, has already led to a marked reduction in neural tube defects by the increase of maternal dietary folate.

Although cardiac muscle defects accompany many types of congenital heart defects, they often arise as secondary effects of a primary disturbance in non-muscle cell lines, such as those of the endothelium and of the neural crest.

Tissue analysis of gene expression with cDNA microarrays provides a measure of the transcriptional or posttranscriptional regulation and cellular recruitment. Large-scale partial sequencing of cDNA libraries to generate expressed sequence tags (ESTs) is an effective means of discovering novel genes and characterizing transcription patterns in different organs and tissues. A part of the whole genome transcripted and handled by a specific tissue is known as transcriptome. The qualitative and quantitative analysis of the genes expressed in cardiac tissue may be of importance for the study of cardiac pathologies.

The assembling of a gene expression data repository and of on-line resources for the retrieval of gene expression data from any organism or artificial source are very helpful tools for the discovery of new genes and tissue specific expression profiles. Many types of gene expression data from platform types such as spotted microarray, high-density oligonucleotide array, hybridization filter and serial analysis of gene expression represent a source for such data sets.

Table I. Molecular pathways for cardiac development. Little is known about the mechanisms that guide the heart tissue across its developmental stages. The genes and proteins here reported seem to play a role in animals and humans.

Developmental stage	Proposed genes or proteins	Postulated function
Cardiac tube formation and looping	Endoderm-derived(?) signaling molecule(s)	Mesoderm commitment into a cardiogenic "field"
	Proliferation and adhesion factors: Shh, Act-RIIa, cNR1, Nkx2.5, eHAND	Cardiac tube formation and looping, looping directionality
Chamber specification and formation	Homeobox genes	Specification of the positional identity
	NCAM, tenascin, other adhesion molecules	Cardiac cushion formation, canal septation
	TGF-β family genes: TGF-β1, TGF-β2, BMP4 Homeobox genes: *msx1*, *mox1*	Atrioventricular valve morphogenesis
	RXRα, N-myc, TEF1, WT1, NF1, α_4 integrin, VCAM	Myocardial growth
	Neuregulin, ERBB2, ERBB4	Ventricular trabeculae formation
Conduction system development	*msx2*	
Neural crest cell migration	TUPLE1, *hoxA3*	Formation of conotruncus and aortic arches
Transcriptional control	Transcription factors: Nkx2.5 (Csx), *mef2*, GATA 4, 5, 6, dHAND, eHAND	Cardioblasts formation and muscle cell differentiation

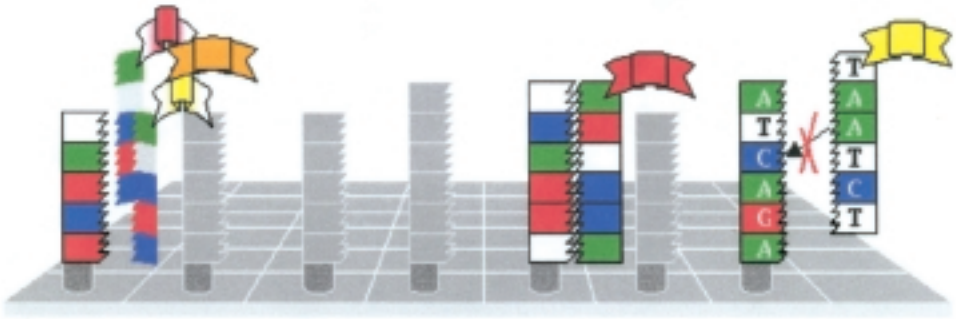

Figure 1. *DNA chip hybridization with two colors target labeling (e.g.* yellow *for normal,* red *for pathological tissue). The interpretation of results is based on the comparison of the luminescent signal intensity.*

When the complete libraries (with no repeated sequences) will be available, single genes should be used for the construction of DNA-drivers. It means that single genes are covalently attached in a mesh format on the glass surface and thereafter hybridized with DNA target sequences. The driver sequence needs to contain all the structural information on expressed genes (or transcripts). Something like 84 904 ESTs are now available, representing more than 26 million nucleotides of raw cDNA sequence data from 13 independent cardiovascular system-based cDNA libraries. Of these, 55% matched to known genes in the GenBank/EMBL/DDBJ databases, 33% matched only to other ESTs, and 12% did not match to any known sequences[5].

When such expression profile studies are planned, it is necessary first to extract RNA from cardiac tissue by traditional methods. Messenger RNA can be copied into cDNA using reverse transcriptase so that the relative abundance of individual mRNAs is reflected in the cDNA product. With further manipulation, a replica of the mRNA expression pattern can be duplicated into a labeled (radioactive or fluorescent) double-stranded DNA probe[6]. The intensity of the hybridization signal for a given gene is the result of its relative abundance in the RNA-derived DNA probe (Fig. 1).

Gene expression microarrays measure the expression levels of thousands of genes simultaneously. They utilize microscopic cDNA elements on a glass surface, low-volume hybridizations of total cDNA and two-color fluorescence detection. In this way, it is possible to obtain both expression patterns, one related to the normal tissue and the other to the pathological tissue simultaneously. Comparing this expression profiles, the interpretation of the results is more immediate.

In conclusion, microarray technology is a revolutionary device for the study of the genetic component of various diseases. It is useful especially for the study of the genetic component in multifactorial diseases as it makes the researcher's job easier.

Although microarray technology has so far been mainly applied in the research setting, its clinical application is expected in the foreseeable future and it is believed that it will lead to the substitution of many obsolete diagnostic procedures.

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