Expression Profiling of the Cardiovascular System by Microarray Technology


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We have initiated applying the DNA microarray technology to the expression profiling of the cardiovascular system in various developmental, physiological and pathological states. The gene expression levels of the mouse hearts have been measured by cDNA microarray (Incyte) and oligonucleotide array (Affymetrix). For more reliable signal intensity values and gene identities, various low-level analyses and corrections of raw data from microarray hybridization have been performed. Based upon these refinements, the transcriptomal analysis that utilizes two-point and multipoint analyses both in combinatorial and novel ways was schematized and performed for the framework of data analysis and interpretation, which are informative of global cellular states or changes, and are harmoniously connected to the follow-up studies with the hypotheses generated. The dataset from the heart undergoing transition from embryonic stage to adulthood and that of a mouse dilated cardiomyopathy model induced by a dominant negative H-Ras in the heart were arbitrarily chosen. The mouse heart undergoing the transition showed a comparatively large transcriptomal alteration, confirming the initial prediction that the cell-division related genes, for example Cdc2, Cdc28, Pcna, be significantly down-regulated. The negative H-Ras mouse model showed a comparatively small change both in gene numbers and in the extent of expression changes, revealing the up-regulation of conventional hypertrophy markers genes, ANF and BNP. Many new genes that have never been characterized and uncharacterized, up to now, in the cardiovascular system showed significant changes in expression levels. Cbfa1, a transcription factor characterized in osteogenesis, was implicated both in the late heart development and the dilated cardiomyopathy. The transcriptomal analysis also revealed that interferon gamma signaling is likely to play important role both in
the cardiac development and in the dilated cardiomyopathy process of the negative H-Ras mouse model.

Figure 1. The coordinate framework for functional genomics data analysis on the developmental (temporal) and pathological (abnormal) states of the heart. 

*ANF* (atrial natriuretic factor) and *BNP* (brain natriuretic protein) are the most well-known markers of the cardiac hypertrophy. The hearts from different time points (the axis for the heart development) and those from different transgenic mouse models of the heart diseases have been subjected to cDNA microarray hybridization to measure the expression levels, fixing the 12-week-old mouse heart (12W) as the reference expression levels (mean absolute expression level (n=8) of *ANF* equals 1956 and that of *BNP* is 739). The relative levels of cardiac mRNA expression at the four time points (E14=embryonic day 14, N1=neonatal, 4W=4 week old, 12W=12 week old) constitute the developmental (temporal) coordinate of expression comparison. The mRNA expression levels of the hearts from five different mouse models consist in the pathological (abnormal) coordinate. The mouse models have incorporated a heart-specifically expressed transgene, for example *dnRas* represents additional copy of the Ha-Ras

![Graph showing relative expression of ANF and BNP across different time points and mouse models.](Image)
modified to produce protein with a dominant negative function.
Figure 2. The developmental expression profiles of some of the cardiovascular genes along the developmental (temporal) coordinate.

a) ANF (1982 = mean expression level at 12 week old, n=8), b) BNP(739), c) Cbfa1/Osf2 (879), collagen alphal (3199), c) Cdc2(468), Cdc28(482), Pcna(890), Cdc42(1382), Cdc47(1122), d) Tim17a(4030), f) Serca1 (12,471), Serca2 (5487). The expression profiles of these genes were arbitrarily chosen for depiction. The mean absolute expression levels of these genes at the hearts of 12 week old mice (the reference point that has been set to 1) are different for each genes, ranging from 468 of the Cdc2 gene to 12,471 of the Serca1 gene. ANF(a) and BNP(b) are the markers of the cardiac hypertrophy. Cbfa1/Osf2 is a transcription factor, whose null mutants are without any bones. Collagen alphal is one of the well-studied downstream target genes regulated by Cbfa1. The representative cell-cycle related genes (Cdc2, Cdc28, Pcna, Cdc42, Cdc47, are downregulated along the heart development coordinate. Tim17a is a protein that is encoded for by the nucleus, but transported onto mitochondria for its proper function. Serca1 and Serca2 are sarcoplasm calcium ATPases in the sarcoplasm reticulum.