Introduction

Heart development is a complex process that requires the step-wise activation of a complex genetic regulatory network. Perturbation of the cardiac regulatory network can result in severe developmental malformations of the heart. Previous published high-throughput gene expression data sets of normal and abnormal mammalian heart development provide the research community with the opportunity to re-investigate important biological processes and develop new hypotheses and experiments.

ZNf503, the mammalian ortholog of the Drosophila nuc gene, encodes a transcription factor that is a putative cardiac regulatory gene and is a gene of interest that is undergoing genic loss-of-function experiments in both Drosophila and mammalian experimental systems (Busser et al., 2015). By using two previously published data sets, we developed a stringent selection criteria to identify downstream cardiac genes that are activated by ZnF53 during cardiomyocyte differentiation.

Method and Discussion

Busser and colleagues (2015) utilized a directed differentiation protocol to differentiate hESCs into cardiomyocytes which generates cardiomyocytes at high efficiencies in a manner that replicates in vivo heart development. Wildtype (WT), Nkx2-5-shRNA knockdown, and ZnF53-shRNA knockdown hESCs cell lines were differentiated into cardiomyocytes. WT hESC differentiated into cardiomyocytes at a high efficiency, but mature cardiomyocytes were not detected in Nkx2-5 and ZnF53 knockdown hESC samples.

Raw RNA-seq high-throughput transcript profiling was evaluated for days 2, 6, and 10 of cardiomyocyte differentiation (Figure 1) using the Illumina HiSeq 2500 sequencing system determining expression values for >41,000 genes.

Figure 1: Cardiomyocyte differentiation of hESCs using a directed differentiation protocol. RNA-seq expression profiling was performed at Day 0, 2, 6, and 10 corresponding to the mesoderm, cardiac progenitor, and cardiomyocyte stage of differentiation (Busser et al., 2015).

Differential expression analyses were performed on this data using a pipeline of Bioconductor packages. To identify significantly changed genes between the WT and shRNA knockdown hESCs, a combination of statistical criteria (Significance of <0.05 with FDR) and fold change criteria of 1.5× was applied to comparisons. Genes significantly down regulated in the ZnF53-shRNA knockdown samples were further filtered on two criteria: 1) Genes must be downregulated in the Nkx2-5-shRNA knockdown samples since Nkx2-5 expression is required for cardiomyocyte differentiation and 2) Human gene orthologs must be upregulated in the mouse early embryonic heart samples. This analysis narrowed the list of candidate cardiac genes positively regulated by ZnF53 to 488 genes (Figure 2). Normalized data was imported into Genespring for data visualization and cluster analysis (Figure 3). All analysis scripts and a more detailed discussion are available at github.com/awilliamson10.

Figure 3: Hierarchical clustering of the 488 genes downregulated in the Day 6 differentiating ZnF53-shRNA knockdown cells compared to wildtype cells. Hierarchical clustering of these genes into 4 distinct expression signatures based on the expression from Day 0 to Day 10. Cluster A and cluster D are the most notable clusters comprised of a high expression gene signature at Day 6 and Day 10 that is reduced from WT levels in the ZnF53 or Nkx2-5 Day 6 samples. A select number of interesting candidate genes are identified in Table 2.

Table 2: The table displays genes identified in each cluster of Figure 3.

Conclusions

This preliminary analysis of the Day 6 ZnF53-shRNA knockdown using these filtering criteria develops a better picture of the cardiac gene network perturbations and potential downstream candidate genes regulated by ZnF53.

Notable observations:

- At differentiation Day 6, both ZnF53 and Nkx2-5 are both downregulated in the shRNA knockdown cells demonstrating effective loss of function.
- Several important cardiac genes were downregulated in the ZnF53 and Nkx2-5 shRNA knockdowns. These genes include both critical cardiac regulatory genes and genetic markers of functional cardiomyocytes identified in Clusters A and D (see Figure 3 and Table 2).
- The genes identified in these analyses will serve as a starting point as we perform our own ZnF53 loss-of-function analysis in our cardiomyocyte differentiation system.

Acknowledgments

SURE Program
The Center for Genomic Advocacy
BD485U, NIH 1R81MD011712-01
Department of Mathematics and Computer Science

References