Epigenetic regulation of cardiomyocyte cyclin A2

Abstract

Heart disease is the leading cause of death in the industrialized world. This is partially attributed to the inability of cardiomyocytes to divide in a significant manner, and therefore the heart responds to injury through scar formation. Indeed, in mammalian hearts, cardiomyocytes proliferate throughout fetal development and into the early neonatal period when division ceases and DNA replication declines quickly. The cessation of myocyte proliferation has been attributed to an arrest of the cell cycle [1]. Cyclin A2 (CCNA2) is a key molecule in both the G1/S and G2/M transitions of the cell cycle and is reported to be silenced in mammalian hearts shortly after birth when cardiomyocyte withdrawal from the cell cycle [2,3].

Our previous work has demonstrated that delivery of CCNA2 encoding CCNA2 induces cardiac regeneration with significant restoration of cardiac function in small and large animal models of myocardial infection [4-6].

We now wish to understand the mechanisms responsible for CCNA2 silencing. We hypothesize that silencing of the CCNA2 promoter hinges on epigenetic modifications that could be targeted in the design of novel therapeutic approaches for cardiac repair.

To identify chromatin remodeling involved in CCNA2 silencing we screened the epigenetic profile of the CCNA2 promoter in two different models: murine cardiomyocytes and human embryonic stem cells (hESCs).

In the murine model we compared the epigenetic status between embryonic cardiomyocytes (where CCNA2 is still active) and adult cardiomyocytes (where CCNA2 is silenced).

In the hESC's model our data indicated that CCNA2 mRNA levels drop abruptly during differentiation to cardiomyocytes while expression levels of PR2 components increase, therefore we proceeded with the assessment of the epigenetic status during cardiac embryo bodies (EBs) formation.

Methods:

• The DNA methylation analysis was performed using the bisulfite modification technique, followed by PCR, cloning and sequencing.
• The histone modification status was evaluated through the use of the chromatin immunoprecipitation (ChIP) technique followed by qPCR.
• hESCs E502 differentiation to cardiac beating EBs: The hESC line, E502, was cultured on mouse embryonic fibroblasts (MEFs) in the presence of NESC media. Prior to differentiation MEFs were depleted by passing the cells onto matrigel coated dishes. When the cells reached 70-80% confluency, EBs were generated from clusters of hESCs cultured on low-adherence dishes. Cardiac differentiation will be induced in suspension culture with various growth factors. Based on our experience with this protocol, EBs show spontaneous beating around day 8-10 of the differentiation protocol.

Conclusions

Our data demonstrates an enrichment of the histone modification repressive mark H3K27me3 in the promoter of the CCNA2 gene in two different models. This points to a potential role for the PR2 complex in the silencing of the gene, as also suggested from data obtained for the maintenance of adult cardiomyocytes.

Around day 7 there is a concomitant upregulation of PR2 components as shown by qPCR.

Analysis of the levels of the most common histone modifications in embryonic versus adult cardiomyocytes showed an enrichment of H3K27me3 in the CCNA2 promoter.

Histone modification

hESCs differentiation to cardiac EBs

PRC2 component expression

Histone modification

Conclusions

Our data demonstrates an enrichment of the histone modification repressive mark H3K27me3 in the promoter of the CCNA2 gene in two different models. This points to a potential role for the PR2 complex in the silencing of the gene, as also suggested from data obtained for the adult cardiomyocytes. This point of view might help to materialize cardiac regeneration in the design of cardiac regenerative therapy and advancing the clinical treatment of heart disease.

These studies may enable the controlled transcriptional reactivation of the CCNA2 gene in the adult cardiomyocyte, and subsequently impact cardiac repair. Elucidating epigenetic silencing mechanisms of CCNA2 is therefore critical in the design of cardiac regenerative therapy and advancing the clinical treatment of heart disease.