MicroRNA profiling of cardiac stem cells to unravel its biologic functions

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Abstract

During the last decade, microRNAs (miRs) have emerged as important regulators of cell differentiation and, in particular, of heart function and response to stress. Cardiac Stem Cells (CSCs) have multiple physiologic properties – proliferation, differentiation, plasticity and cell fate determination - that are influenced by miRs in other biologic systems, as hematopoiesis, embryonic stem cells or heart development. However, the identification of the miRs present in CSCs and their role in the establishment of those properties has not been systematically evaluated. The enormous potential of miRs to exogenously control different biologic systems has not escaped both the academic and corporate communities who are presently applying enormous research efforts to the development of therapeutic applications.

We have developed a unique protocol to isolate without digestion CSCs by soft mechanical destruction of mouse cardiac tissue. This has allowed us to profile CSC miR that otherwise would be digested. Comparative analysis of miRs expressed in CSCs, embryonic heart cells (E9.5) and adult mesenchymal stem cells using a dedicated stem cell and differentiation focused miR array, allowed us to identify a signature miR profile specific of cardiac SCs, that correlates to their distinctive biological properties. We have found that CSCs display a mixed miR expression profile reflecting their similarities and differences with the two other cell types. This study provides a pioneer insight into the miR-dependent gene expression networks that are active in CSCs, establishing novel and testable hypothesis regarding the mechanisms that regulate their distinctive biological properties to fit.

Methods

Target cell population: Adult heart progenitor cells

1. Cell isolation

Tissue dissociation + FACS sorting (<0.1% of cells in sample)

2. Total RNA/microRNA isolation

miRvana RNA isolation kit + quantification and quality control with Bioanalyzer picotag 6000 RNA chip

3. microRNA expression profiling

qPCR array for stem cell miRs (System Biosciences) + Taqman qRT-PCR probes for selected miR/small RNAs

qPCR array for microRNA expression profiling

To establish the microRNA expression profile in the target cell population, a quantitative real-time PCR array method was used - the Stem Cell MicroRNA qPCR Array with Quantimir (SBi). This array includes probes for 95 known miRNAs and for the U6 snRNA transcript as a housekeeping gene for normalization. The microRNAs that were chosen to integrate this array are known to be involved in several processes, have potential roles in stem cell self-renewal, hematopoiesis, neuronal development and differentiated tissue identification. For comparative purposes, quantification of miR expression in lineage negative mouse mesenchimal stem cells (panel B), which have been described to be able to differentiate into heart cell types, and embryonic heart cells (day E9), which are actively engaged in the proliferation and cardiac differentiation program, were also performed.

Conclusions

Comparative microRNA expression profiling

To obtain insights into the origins and gene expression signatures of Sca1 positive mouse adult cardiac stem cells, we have performed a comparative profiling of microRNA expression in three independent total RNA samples from isolated cardiac stem cell (CSC1 to 3), bone marrow mesenchymal (lineage negative) stem cells (BM1 to 3) and mouse embryonic heart, day E9 (EMB1 to 3). All these cell types are known to give rise to the cell populations present in the adult heart, but differ significantly in their commitment to cardiac fate and proliferation activity. Hierarchical clustering of miR profiles and samples was performed to obtain an overview of signature profiles for each sample type.

We find that adult cardiac stem cells display an overall distinctive miR expression profile that can differentiate them from the other two populations used in this study. A subset of miRs (box B) is expressed at higher levels in cardiac stem cells (CSC) when compared to the other two populations. However, these cells also express subsets of miRs that are common either to bone marrow and embryonic heart cells (box A and C, respectively). Finally, our results identify a group of miRs with high expression levels in both embryo and bone marrow cells, but which display very low levels of expression in adult cardiac stem cells (box D).

Disclosure of Interest: The Authors have no conflict of interest to disclose