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PRECLINICAL RESEARCH

Cardiomyocyte Lineage Specification in Adult Human Cardiac Precursor Cells Via Modulation of Enhancer-Associated Long Noncoding RNA Expression

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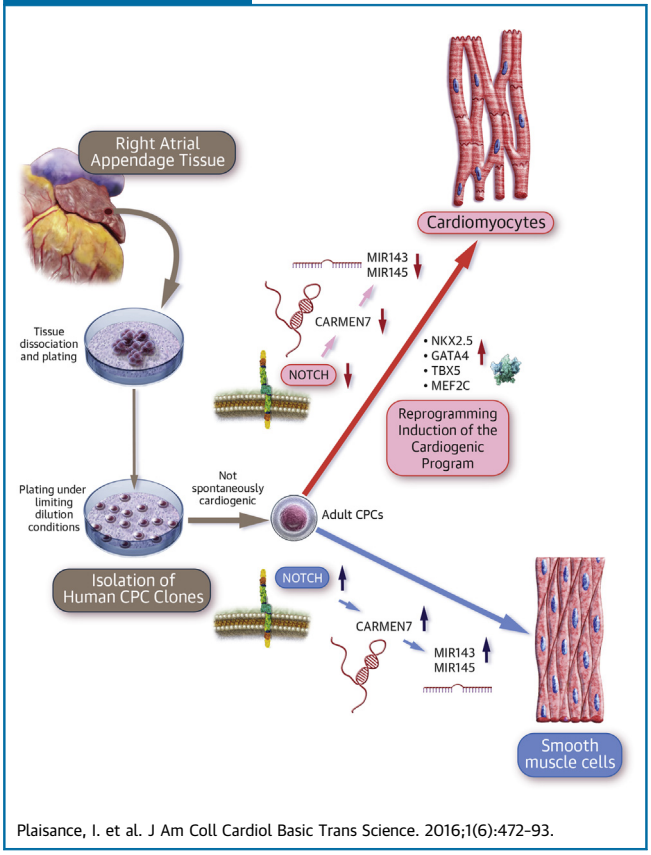
VISUAL ABSTRACT



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HIGHLIGHTS

- **Human CPCs produce predominantly smooth muscle cells.**
- **CPCs can be redirected to the cardiomyocyte fate by transient activation followed by inhibition of NOTCH signaling.**
- **Inhibition of NOTCH signaling during differentiation represses *MIR-143/145* expression and blocks smooth muscle differentiation.**
- **Expression of the microRNAs is under control of *CARMEN*, a long noncoding RNA associated with an enhancer located in the *MIR-143/145* locus and target of NOTCH signaling.**
- **The *CARMEN/MIR-145/143* locus represents a promising therapeutic target to favor production of cardiomyocytes in cell replacement therapies.**

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SUMMARY

The mechanisms controlling differentiation in adult cardiac precursor cells (CPCs) are still largely unknown. In this study, CPCs isolated from the human heart were found to produce predominantly smooth muscle cells but could be redirected to the cardiomyocyte fate by transient activation followed by inhibition of NOTCH signaling. NOTCH inhibition repressed *MIR-143/145* expression, and blocked smooth muscle differentiation. Expression of the microRNAs is under control of *CARMEN*, a long noncoding RNA associated with an enhancer located in the *MIR-143/145* locus and target of NOTCH signaling. The *CARMEN*/*MIR-145/143* axis represents, therefore, a promising target to favor production of cardiomyocytes in cell replacement therapies. (J Am Coll Cardiol Basic Trans Science 2016;1:472–93)

The adult human heart has poor regenerative potential, and heart failure gradually develops following injury (1,2). Ultimately, heart transplantation represents the only therapeutic option for end-stage heart failure. Within this context, stimulation of cardiomyocyte production in the damaged heart to promote regeneration represents an attractive therapeutic approach (3,4). In particular, cell replacement therapy via injection of precursor cells into the damaged heart represents an interesting therapeutic avenue. The main challenge for transferring cell therapies for heart disease into a clinical setting is to identify a suitable source of human cardiac precursor cells (CPCs). Direct isolation of CPCs from the heart of cardiac patients would represent a great advantage by the autologous nature of the isolated cells. This would indeed reduce the problems associated with immune rejection. The existence of resident CPCs in the adult mammalian heart, including the human heart, capable of differentiating into functional cardiomyocytes, has been demonstrated (3,4). However, the number of CPCs in the adult myocardium is quite low, and isolation of these cells is a challenging procedure. Indeed, no truly specific markers are currently available to distinguish CPCs from other cell types (3,4). Multipotent mesenchymal stromal cells expressing cardiac transcription factors such as GATA4, NKX2.5, and MEF2C, but no proteins expressed by fully differentiated cardiomyocytes such as proteins of the sarcomere, could therefore be operationally defined as CPCs. Nevertheless, the effective generation of new cardiomyocytes from transferred CPCs is still a matter of intense debate, and restoration of function has been attributed to paracrine mechanisms mediated by factors secreted from the transferred cells (2–4). Therefore, a clear understanding of the regulatory networks controlling mobilization and differentiation of endogenous CPCs toward the cardiac lineage is required in order to facilitate the ultimate goal of cardiac regeneration.

Several pathways that are important during cardiac morphogenesis are reactivated in the damaged myocardium. Among these, the NOTCH pathway plays crucial roles in the developing and adult heart (5,6). NOTCH is an evolutionarily conserved cell-to-cell communication system that takes place between 2 adjacent cells (7). The signal-sending cell expresses a membrane-bound ligand such as Jagged (J)1, J2, Delta-like1 (DLL1), DLL3, and DLL4, and the signal-receiving cell expresses a NOTCH receptor such as NOTCH (N)1, N2, N3, and N4. Receptor engagement results in its cleavage and liberation of the NOTCH intracellular domain (NICD). NICD translocates into the nucleus, where it interacts with co-activators, in particular a transcription factor known as RBPJ, to activate target gene expression. NOTCH target genes include repressors of the Hairy enhancer of split (*HES*) and the related *HEY* families (8). During development, NOTCH regulates trabeculation, myocyte proliferation, and valve formation. In the neonatal heart, NOTCH controls cardiac precursor expansion and differentiation (9). In the adult heart, NOTCH signaling is activated in cardiomyocytes, CPCs, and fibroblasts (10–14). Interestingly, NOTCH appears to prevent premature cardiogenic differentiation in precursor cells, and to favor proliferation in this transient amplifying cell compartment (14). Consistent with this observation, blockade of the NOTCH pathway in embryonic stem cells favors commitment into the cardiac mesoderm, and subsequently, into cardiomyocytes, at the expense of the neuroectodermal lineage (15). NOTCH signaling has also been reported to induce early cardiac commitment in embryonic and induced pluripotent stem cells, supporting a biphasic role of NOTCH in cardiogenesis (16). Accordingly, NOTCH signaling

ABBREVIATION AND ACRONYMS

CARMEN = (CAR)diac (M)esoderm (E)nhancer-associated (N)oncoding RNA

CPC = cardiac precursor cell

DLL1 = Delta-like1

GO = gene ontology

J1 = Jagged1

lncRNA = long noncoding RNA

miRNA = microRNA

NICD = NOTCH intracellular domain

RT-PCR = reverse transcription polymerase chain reaction