The role of charme in the phenotype and function of resident cardiac mesenchymal stromal cells

E. Floris\textsuperscript{1}, V. Picchio\textsuperscript{1}, C. Cozzolino\textsuperscript{1}, V. Taliani\textsuperscript{1}, G. Buonaiuto\textsuperscript{1}, C. Nicoletti\textsuperscript{1}, M. Ballarino\textsuperscript{1}, I. Chimenti\textsuperscript{1}, F. Pagano\textsuperscript{2}

\textsuperscript{1}Sapienza University of Rome, Rome, Italy
\textsuperscript{2}Centro Nazionale delle Ricerche, Rome, Italy

\textbf{Funding Acknowledgements:} Type of funding sources: Public Institution(s). Main funding source(s): Sapienza University of Rome - Progetti per Avvio alla Ricerca - Tipo 1

\textbf{Background:} Charme is a murine long non-coding RNA with specific expression in striated muscles, presenting a human orthologue with 45\% identity. Its depletion leads to incomplete muscle differentiation. Charme-/- mice display an altered cardiac phenotype with increased wall thickness and reduced chamber dimensions due to myocyte hyperplasia. Left ventricular dilatation and reduced fractional shortening are detected in aged Charme-/- mice. Embryonic myocardial architecture reveals aberrant compaction and ventricular hypotrabeculation, resembling some congenital cardiomyopathies. Cardiac mesenchymal stromal cells (CMSCs) play key roles in tissue homeostasis and pathophysiology, by maintaining the extracellular matrix and by paracrine action on all myocardial cells.

\textbf{Purpose:} To elucidate the phenotype, paracrine function, and myofibroblast differentiation capacity of resident CMSCs in the Charme-/- mouse model.

\textbf{Methods:} CMSCs were isolated from 5-week-old wild-type (WT) and Charme-/- mice by explant culture, pooling at least 4 different hearts per culture.

\textbf{Results:} Charme-/- CMSCs showed increased features of primitive phenotypes, such as enhanced spontaneous spheroid growth (123.6±11.9 vs 51.0±6.4 spheroids/well; N=8, \(p<0.0001\)) and clonogenesis efficiency (2.7±0.5-fold; N=7, \(p<0.05\)) compared to WT cells. Charme-/- CMSCs also showed increased migration ability (Charme-/-: 68.5±2.7\% vs WT: 80.2±0.7\% wound area; N=2). Flow cytometry identified a reduced proportion of lin-/Sca1+/CD90+ primitive mesenchymal cells in Charme-/- versus WT CMSCs (7.4±1.6\% vs 56.9±6.5\%; N=6, \(p<0.05\)). Western blot analysis on Charme-/- whole cardiac tissue showed reduced collagen I and collagen I/III protein ratio compared to WT hearts (0.17±0.03 vs 0.85±0.10 normalized OD; N=4, \(p<0.005\)). Stimulation with TGFβ-1 resulted in lower expression of fibroblast activation markers compared to WT CMSCs, as assessed by immunofluorescence staining for aSMA (0.49±0.05 vs 0.76±0.10 mean fluorescence intensity; N=6-12, \(p<0.05\)) and collagen I/III mRNA expression ratio (1.3±0.1 vs 2.2±0.4 2^-DCt; N=3, \(p<0.01\)). The secretome of Charme-/- CMSCs revealed a general depletion in many cardioprotective cytokines, included in the KEGG term “PI3K-Akt signalling pathway” (FDR<0.0005) and classified in Gene Ontology categories of myoblast differentiation and fusion (GO:1901739, GO:1901741, GO:0045663, GO:0045661; FDR<0.05). In line with this, the secretome of TGFβ1-stimulated Charme-/- CMSCs was less effective in sustaining cardiomyocyte survival versus the WT CMSC secretome (1.70±0.03 vs 1.90±0.04 normalized OD at 48h; N=5, \(p<0.005\)).

\textbf{Conclusions:} Charme-/- CMSCs show a less mature phenotype associated to reduced collagen I presence in situ, reduced activation and differentiation upon stimulation, and impaired cardioprotective paracrine functions, suggesting a key role of Charme in the physiology of the cardiac stroma, possibly mediating in part the altered cardiac phenotype of Charme-/- mice.