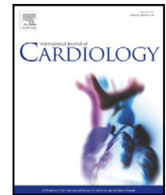
International Journal of Cardiology 216 (2016) 140–150

Contents lists available at ScienceDirect

International Journal of Cardiology

journal homepage: www.elsevier.com/locate/ijcard



Critical role of lysosomes in the dysfunction of human Cardiac Stem Cells obtained from failing hearts☆

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ARTICLE INFO

Article history:
Received 6 November 2015
Received in revised form 10 April 2016
Accepted 20 April 2016
Available online 26 April 2016

Keywords:
Stem cells
Heart failure
Lysosome
TFEB
Cell senescence

ABSTRACT

The in vivo reparative potential of Cardiac Stem Cells (CSC), cultured from explanted failing hearts (E-), is impaired by cellular senescence. Moreover, E-CSC are characterized, with respect to CSC obtained from healthy donors (D-), by an arrest in the autophagic degradation. Although the lysosome plays a pivotal role in cellular homeostasis and defects of this organelle may be associated with aging and heart failure, the lysosomal function of CSC has never been investigated. The aim of this work was to focus on the Lysosomal Compartment (LC) of E-CSC, evaluating elements that could jeopardize lysosome functionality.
Methods and results: Bioinformatics analysis conducted on genes differentially expressed between D- and E-CSC identified lysosomal-related gene sets as significantly enriched. Moreover, 29 differentially expressed genes were part of CLEAR (Coordinated Lysosomal Expression and Regulation) gene network, by which Transcription Factor EB (TFEB) regulates cellular clearance. Consistently, live cell imaging and flow cytometry analyses showed that the lysosomes of E-CSC are less acidic than the D-CSC ones. Furthermore, confocal microscopy showed in E-CSC: an accumulation of intralysosomal lipofuscins, a reduction of cathepsin B activity, evidence of lysosome membrane permeabilization, and the reduction of the nuclear active TFEB. The use of Rapamycin (TORC1 inhibitor) was able on one hand to increase TFEB activation and, on the other hand, to reduce lipofuscin mass, potentiating the lysosomal functionality.
Conclusions: This study demonstrated for the first time that E-CSC are characterized by a blunted activation of TFEB and an altered proteostasis. TORC1 hyperactivation plays a central role in this phenomenon.

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Abbreviations or acronyms

CSC	Cardiac Stem Cells
D-CSC	Cardiac Stem Cells isolated from Donor hearts
E-CSC	Cardiac Stem Cells isolated from Explanted, failing hearts
HF	Heart failure
LC	Lysosomal Compartment
LV	Left ventricle
AO	Acridine orange

1. Introduction

Cellular senescence is a cellular condition characterized by a stable arrest of the cell cycle coupled with stereotyped phenotypic changes

☆ Grant support: Project ERC-7FP SP 2 IDEAS QUIDPROQUO G.A. n. 269051 (G.S., D.C., A.P.B., G.G.). Italian Ministry of Research and Technology, FIRB accordi di programma 2011 n. RBAP11ETKA_007 (A.P.B). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript.
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http://dx.doi.org/10.1016/j.ijcard.2016.04.155
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[1]. Increasing evidence shows a cause–effect correlation among cellular senescence, aging, and cardiovascular disease, including heart failure (HF) [2]. This correlation involves human Cardiac Stem Cells (CSC) too. Indeed, we recently demonstrated that senescent CSC isolated from explanted ischemic hearts (E-CSC) are unable to protect in vitro rat adult cardiac myocytes, exposed to Simulated Ischemia/Reoxygenation (SI/RO), from apoptosis and senescence. When compared with healthy donor heart-derived CSC (D-CSC), E-CSC are characterized by a blunted ability to stimulate a reparative healing of the infarcted mouse heart [3]. By analyzing the molecular pathways connected with CSC senescence, we found, inter alia, that the hyperactivation of the mTOR Complex 1 (mTORC1), followed by a block in the autophagic flux, plays a crucial role in the dysfunction of E-CSC [3]. However, the specific contribution played by the Lysosomal Compartment (LC) in the senescence of this cell type has not been elucidated yet.

The LC is considered to be the final destination of every intracellular degradative pathway (i.e. autophagy, endocytosis, and phagocytosis), with the only exception of the ubiquitin-proteasome system [4]. Lysosomes are eukaryotic, membrane-enclosed, organelles, containing more than 80 different acid hydrolases (i.e. proteases, lipases, nucleases, glycosidases, phospholipases, phosphatases and sulfatases) that are able to degrade an array of biological polymers at an optimal pH of 4.5. This latter is guaranteed by proton pumps (vacuolar ATPase) that reside in lysosomal membranes and is responsible for maintaining the concentration of intralysosomal H^+ 100-fold higher than that of cytosol [4,5]. Besides the degradation, lysosomes are also responsible for other relevant activities including: programmed cell death [6] and exocytosis. This latter is useful to eliminate toxic substances from the cell [7], to release cellular products such as cytokines [8], and to repair the plasma membrane in both normal and pathological conditions [9].

Given that the cellular needs of lysosomal activity significantly change as a function of variables such as the tissue type, the organism age, and the environment, many lysosomal genes exhibit coordinated behavior and are regulated by Trascription Factor EB (TFEB) [10]. This latter is one member of the microphthalmia Transcription Factor (Mit/TFE, subfamily of transcription factors basic Helix–Loop–Helix, bHLH, leucine zipper) family, and is a master gene that positively regulates genes belonging to the CLEAR (Coordinated lysosomal Expression and Regulation) network targeting, among others, genes encoding for lysosomal hydrolases, lysosomal membrane proteins and components of the vacuolar H^+-ATPase (V-ATPase) [10]. To date, 471 genes are identified as TFEB direct targets and represent essential components of the CLEAR network [11]. The study of the functional meaning of these genes clarified that TFEB, through the CLEAR network, regulates lysosomal biogenesis, lysosomal degradation proficiency, autophagy, and other lysosomal-related functions, such as exocytosis, endocytosis, phagocytosis and immune response [11]. In this manner TFEB controls all the paths in which lysosomes are involved in cellular clearance [7,10,11,12,13]. TFEB participates also to a lysosome adaptation mechanism that enables cells to respond both to nutrient conditions and to lysosomal stress by interacting with mTORC1 on the lysosome membrane. Indeed, in the absence of lysosomal stress and in nutrient rich conditions, activated mTORC1 is recruited to the lysosome surface, where it transiently binds to and phosphorylates TFEB, inhibiting its nuclear translocation [14].

Growing evidences report that animal health- and life-span are related to the ability of organisms to respond to stress [15]. Specifically the capability of cells to prevent senescence depends on their efficiency in avoiding the accumulation of damaged macromolecules and organelles and so, on their LC [16]. In line, a defect in the Lysosomal Associated Membrane Protein 2 (LAMP2) is characterized by muscle weakness and cardiomyopathy in mice and Danon disease in patients [17]. Moreover, aging is accompanied by a reduced renewal of cardiomyocytes due to a reduction in both the differentiation of stem cells into cardiac myocytes, and in the division of these latter [18,19]. As a result, undigested lipids, metals, and oxidatively modified proteins accumulate in the cells, in the form of autofluorescent pigments (lipofuscins), which are hardly degraded by the lysosomal hydrolases. The age-related deposition of lipofuscin progressively reduces degradative potential of lysosomes, reducing their ability to fuse with autophagosomes, thus impairing the autophagic flux [20]. Specifically, aged, lipofuscin-rich cardiac myocytes become overloaded with damaged mitochondria, leading to increased oxidative stress, apoptotic cell death, loss of myocardial tissue, and finally development of HF [21]. As a consequence, the proper operation of LC appears to be crucial in the progression of aging.

For the reasons described above, the aim of the present study was firstly, to monitor the efficacy of LC in senescent CSC isolated from end-stage failing hearts. After the demonstration that a dysfunctional LC characterized these cells, we identified the causes of this dysfunction and finally tested a drug-based strategy to interfere with them.

2. Materials and methods

2.1. Patient enrollment and ethical approval

In this study, patients with end-stage heart failure (Stage D AHA classification) who underwent cardiac transplantation, at the University Hospital of Udine, were enrolled as in [3].

The study was approved by the Ethics Committee of Udine (2 August 2011, reference number 47831) in accordance with the Declaration of Helsinki and written informed consent was obtained from each enrolled patient.

2.2. CSC isolation, culture and treatment

Atrial samples were collected both from donor hearts (n = 14) and from explanted hearts (n = 13) of ischemic patients undergoing cardiac transplantation at the University Hospital of Udine. Table 1 summarizes the demographic and anatomic characteristics of enrolled subjects.

Human CSC were isolated, as in [22,23]. Specifically, atrial samples were first minced with a scalpel. Subsequently, myocardial fragments (≈ 1 mm^3 in size) were enzymatically dissociated incubating them in a 0.25% Collagenase type II solution (Worthington Biochemical Corporation) in Joklik modified Eagle's Medium (Sigma-Aldrich) for 15–20 min at 37 °C. The enzyme activity was stopped by the addition of 0.1% bovine serum albumin (BSA, Sigma-Aldrich) solution in Joklik modified Eagle's Medium (Sigma-Aldrich). The cell suspension was first centrifuged at 100 g for 1 min to remove myocytes and subsequently at 500 g for 5 min. The resuspended pellet was filtered through a pre-wet strainer whose pore size is 40 μm (BD Falcon). The filtered suspension was centrifuged at 500 g for 5 min. Pelleted cells were finally plated at a concentration of $1.5 \cdot 10^6$ in a 100-mm dish and subcultured as in [3]. All the experiments were done at the fourth passage in culture.

Table 1
Demographic and anatomical data of the 27 enrolled subjects. *Data are expressed as mean ± SEM. LV = left ventricle. N.S. = not significant.

	Explanted patients	Controls	*p*
Sample size (n)	13	14	
Age (years)*	64 ± 7	49 ± 11	<0.0001
Sex (male/female)	11/2	7/4	N.S.
Heart weight (g)*	519 ± 88	–	–
Inner LV diameter (cm)*	10 ± 95	–	–
External transverse diameter (cm)*	13 ± 0.9	–	–
Inner longitudinal LV diameter (cm)*	10 ± 0.9	–	–
Anterior LV thickness (cm)*	1.1 ± 0.6	–	–
Lateral LV thickness (cm)*	1.2 ± 0.3	–	–
Posterior LV thickness (cm)*	0.9 ± 0.5	–	–
Septum thickness (cm)*	1.0 ± 0.4	–	–

Summary of the main demographic characteristics of the 27 subjects whose hearts were included in the study. For the explanted patients, anatomical data of the explanted organs, collected by an expert pathologist at the time of transplantation, are also provided. The column *p* indicates the significance of the comparison of age and gender distributions between patients and controls.

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