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Original article

Stem cell markers in the heart of the human newborn

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Abstract

The identification of cardiac progenitor cells in mammals raises the possibility that the human heart contains a population of stem cells capable of generating cardiomyocytes and coronary vessels [1]. Several recent studies now show that the different cell types that characterize the adult human heart arise from a common ancestor [2, 3]. Human cardiac stem cells differentiate into cardiomyocytes, and, in lesser extent, into smooth muscle and endothelial cells. The characterization of human cardiac stem cells (CSCs) has important clinical implications. In recent years, CD117 (c-kit) has been reported to mark a subtype of stem/progenitor cells in the human heart, with stem cell-like properties, including the ability to self-renewal and clonogenicity multipotentiality.

Keywords

Stem cell, markers, heart, newborn.

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Introduction

Cardiovascular diseases are still one of the most important public health problems in Italy, as well as in the vast majority of developed countries. Heart failure represents the leading cause of morbidity and is the leading cause of death in industrialized countries, with about 16.7 million deaths per year. Included in this group, a relevant role is played by the most frequent diseases of atherosclerotic origin, in particular ischemic heart diseases (acute myocardial infarction and angina pectoris).

One who survives an acute heart attack becomes a chronic sufferer, with considerable consequences on the quality of life, and with relevant economic and social costs. The extent of the problem and the limitation in the management of chronic cardiac patients are of great interest with regard to cardiac regenerative medicine [4].

The original inability to distinguish mitosis in the nuclei of cardiomyocytes was at the basis of the theory that the adult heart might consist of a homogeneous population of fully differentiated cells with the chance of suffering from hypertrophy in case of an increase in cardiac work.

In different cardiac pathologies, there is loss of cardiomyocytes: this pathological change is generally compensated by an increase in the size of the surviving cells. However, the response becomes quickly inadequate and hypertrophic cells fail to perform efficiently their own function.

In recent years, numerous studies have been carried out in experimental models and in the human heart, suggesting the persistence of stem/progenitor cells in the adult heart. Moreover, cardiac stem/progenitor cells have been shown to potentially undergo differentiation into cardiomyocytes, through different molecular mechanisms, including the reactivation of fetal molecular programs [5].

Experimental studies have shown that the cellular microenvironment has a crucial role to support the persistence of stem cells and in cell differentiation [6]. Undifferentiated cardiac stem cells grow in peculiar stem cell niches named "cardiospheres".

Cardiac stem cells dividing asymmetrically give origin to daughter cells which correspond to colony forming cells (CFCs). The latter retain a certain plasticity of development and, in case of need, can occupy an empty niche, becoming resident stem cells. CFCs undergo a finite number of divisions; as a result of each division, the replicative capacity gradually decreases and, after a certain number of divisions, CFCs differentiate into mature heart cells [7]. The niches of CSC are mainly found in the courts and in the apex, two cardiac zones that are considered anatomically protected areas, being characterized by low hemodynamic stress, low oxygen tension and therefore by a reduced metabolism [8-13].

Cardiac stem cells are found inside the niches, which are typically found in depth away from exogenous stimuli [14-16]. The niche is a dynamic entity in which the control of stem cell function depends on the complex interaction among intrinsic and extrinsic factors. Stem cells are defined by their behavior, rather than by specific genes they express. The set of genes that distinguishes stem cells from the cell progeny has not been well defined yet [17].

Inside the cardiac stem cell niches, the behavior of stem cells is modulated by physical and chemical signals, including cytokines and adhesion molecules expressed on the progenitor cell surface, cutting forces, voltage oxygen, innervation, and the ions. These are the main determinants of the function of cardiac stem cells [18, 19]. Multiple supporting cells, including stromal cells, play a relevant role and mediate a variety of effector fate of stem cells within the niches, promoting stem self-renewal or encouraging their migration and differentiation. The two-way communication between stem cells and supporting cells is crucial, influencing the state of quiescence or of activation of heart stem/progenitor cells [20].

The importance of the microenvironment inside the cardiac stem cell niche has been recognized shortly after the discovery of this class of stem cells. The relevant role played by the niche is well underlined by the finding that, in the absence of well organized niches, the primitive cardiac stem cells cannot be stored. Outside of their natural environment, cardiac stem cells acquire a high probability to differentiate, a process that can lead to exhaustion of the stem cell compartment.

Objectives

The main purpose of the study was to look for the presence of cardiac stem and progenitor cells in the human heart, in the early phases of the intrauterine

development, and verify if stem/progenitor cells were present in higher amounts as compared to the adult heart.

The main goals that we set at the beginning of the research may be summarized as follows:

- the verification of the presence of progenitor cells in the human heart during gestation;
- the location of the niches of cardiac stem cells in the fetal heart and in preterm babies;
- the definition of immunohistochemical markers of human cardiac stem/progenitor cells.

Patients and methods

For this study, we used heart samples obtained from nine subjects, six fetuses of different gestational ages, ranging from 11 up to 21 weeks, and three preterms of 29, 29 and 34 weeks, respectively. All fetuses and newborns participating to this study did not show any congenital malformation. The procedures performed in this study were approved by the local ethical committee of the University of Cagliari in accordance with the protocols of the Declaration of Helsinki.

For each fetus, section of the thickness of 3-4 μm were obtained. The morphological study of heart samples was mainly based on the histological study of hematoxylin and eosin-stained sections. Tissue sections were accurately scanned at high power (400-630 X) by two pathologists (AF and EP), in order to detect the presence of possible stem cell niches in the developing cardiac tissue.

In each heart sample, the following immunohistochemical markers were utilized, based on data derived from the literature. The markers used in our study were: CD44, CD117 (c-kit), Isl1, WT1 and Wnt1.

Results

At histology we examined the nine fetal hearts in different areas (epicardial, subepicardial, intramyocardial, and subendocardial regions), in order to detect the presence of stem cell niches. By this approach, we were able to reveal the presence of stem cell niches in all the nine hearts examined. They were mainly localized in the subepicardial regions, in strict contact with the overlying epicardium. Cardiac stem/progenitor cells appeared as large cells, with oval nuclei, scant cytoplasm and indistinct cell borders. They were arranged in solid nests, occasionally showing a tendency to aggregate in an onion-like arrangement around a solid central cell group. Stem/progenitor cells were in strict contact with the overlying pericardium. Occasionally, a small vessel was found in close proximity to the stem cell nests. At the deeper front of stem cell niches, it was possible to detect intermediate cells between stem/progenitor cells and cardiomyocytes, probably representing intermediate stages of differentiation of cardiac stem cells committed toward the cardiomyocyte lineage.

When the density of stem cells was compared among the nine fetuses and newborns analyzed in this study, a marked variability was found regarding the number and the size of stem cell niches and the number of cardiac stem cells in the subepicardial area. In the hearts of fetuses and newborns with more advanced gestational age, the number of niches decreases significantly, suggesting a progressive exhaustion, or better reduction, of cardiac stem cell burden during gestation. Stem cell niches were detected even in the heart of the oldest newborn, suggesting the persistency of a pool of stem/progenitor cells persisting even in the perinatal period and probably representing a stem cell reserve pool.

Stem cells and stem cell niches were not restricted to the subepicardial zone. Nests of cardiac stem/progenitor cells were also found scattered among the cardiomyocytes, showing the same morphology previously described in the subepicardial niches. Regarding the size of the intramyocardial stem cell niches, they were smaller and formed by a reduced number of stem/progenitor cells. No well-defined border was found between the deep niche and the surrounding cardiomyocytes. Contrary to the subepicardial niches, the density and the size of intramyocardial niches did not decrease during gestation. In the majority of hearts examined, we observed a mild increase in the number of intracardiac stem cell niches, suggesting the existence of a switch between subepicardial and intramyocardial niches at birth.

Conclusions

Characterization and identification of cardiac stem cells in humans may have important clinical implications in terms of regenerative medicine with the hope of a cure in patients with chronic cardiovascular disease.

In this study, we clearly showed that the fetal and neonatal human heart represents a very useful model for the study of cardiac stem cells. First of all, we found a high number of stem/progenitor

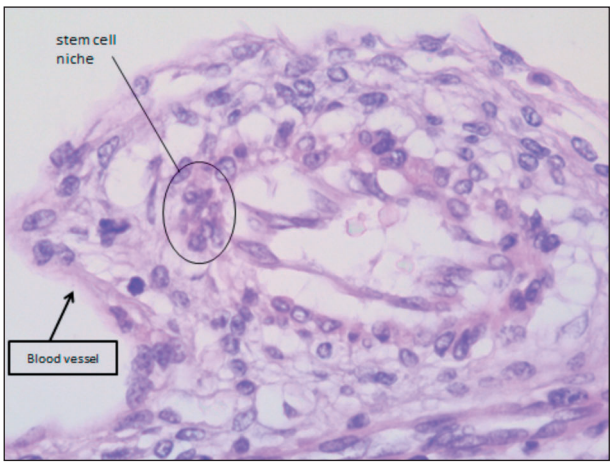

Figure 1. 11-week fetus, H&E staining, subepicardial vessel, stem cell niche.

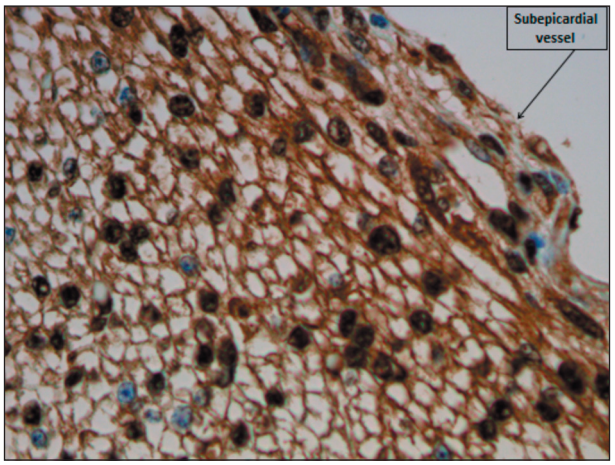

Figure 2. 11-week fetus, diffuse ISl1 immunopositivity with cytoplasmatic and nuclear positivity.

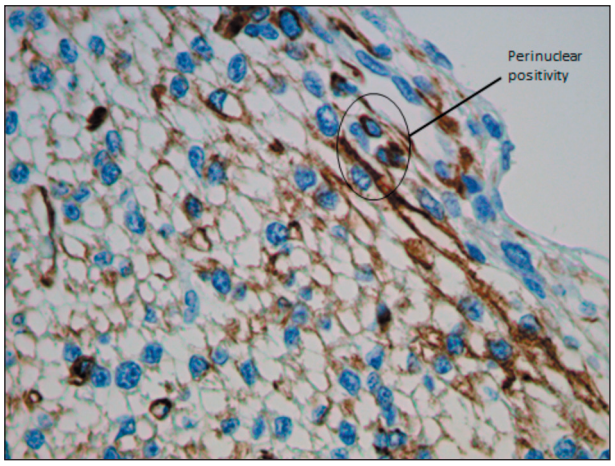

Figure 3. 11-week fetus, subepicardial region, diffuse WT1 immunopositivity with cytoplasmatic and perinuclear positivity.

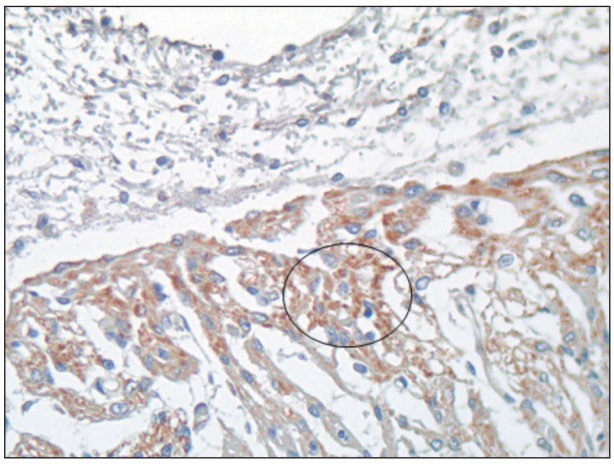

Figure 4. 20-week fetus, subepicardial region, Wnt1 immunopositivity with cytoplasmatic and perinuclear positivity.

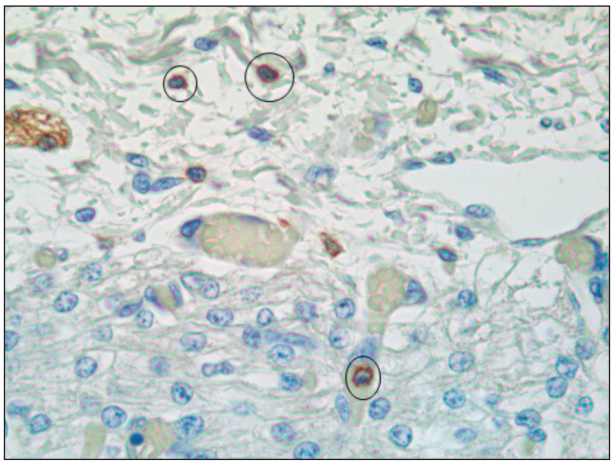

Figure 5. 21-week fetus, subepicardial region, CD44 perinuclear immunopositivity.

cells in all the hearts analyzed. The huge amount of stem cells represents a source for future studies, aimed at identifying the markers expressed by cardiac progenitors that will be useful for their identification in the adult heart in which only very few stem cells persist.

Another interesting finding emerging from our study is the definition of the cardiac stem cell niches. The niches appeared as large and complex structures, mainly formed by undifferentiated stem/progenitor cells, but with the presence of other cell types, including vascular and stromal cells. Moreover, niches were strictly bound to the surrounding pericardium and to the neighboring cardiomyocytes, so that pericardial cells shoud be included in the composition of the subepicardial stem cell niches. As a consequence, the composition

of the cardiac stem cell niche appears, on the basis of our data, complex and not well defined yet. Further studies, mainly based on immunohistochemistry, are needed, in order to better characterize all the cell types participating in the stem cell niche architecture and to better define their function in maintaining the stemness or, alternatively, in favoring the differentiation process.

Other than in the subepicardial zone, stem cell niches were also found inside the myocardium, in strict contact with the developing cardiomyocytes. The presence of these deep cardiac stem cell niches is a new finding, and suggests the existence of multiple niches in the developing human heart.

An intriguing finding is represented by the variability of the cardiac stem/progenitor niches during gestation. Whereas the subepicardial niches progressively decreased during gestation, intramyocardial niches increased their frequency. This represents a new finding that deserves further studies in a large series of fetal and neonatal hearts. A possible switch at birth between the two types of niches may be hypothesized, suggesting a major role for intramyocardial niches in heart development after birth, and a major role of subepicardial niches in the intrauterine life.

Finally, the study of the fetal human heart, when focused on the cardiac stem cells, may represent, in our opinion, a relevant tool for better understanding the human embryonic development and organogenesis. In this context, we might significantly improve our knowledge in the field of cardiac regenerative medicine, which uses mechanisms and cells responsible for organogenesis in order to repair the cardiomyocyte damage caused by heart disease and aging. A better knowledge of the mechanisms regulating the function of cardiac stem cells during development will allow researchers involved in cardiac regenerative medicine to learn how to stimulate adult stem cells during ischemia, inducing the reconstitution of the myocardium, ending with the recovery of the cardiac function [21].

Declaration of interest

The Authors declare that there is no conflict of interest.

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