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Review article

The cardiac fibroblast: Origin, identity and role in homeostasis and disease

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ABSTRACT

The mammalian heart is responsible for supplying blood to two separate circulation circuits in a parallel manner. This design provides efficient oxygenation and nutrients to the whole body through the left-sided pump, while the right-sided pump delivers blood to the pulmonary circulation for re-oxygenation. In order to achieve this demanding job, the mammalian heart evolved into a highly specialised organ comprised of working contractile cells or **cardiomyocytes**, a directional and insulated **conduction system**, capable of independently generating and conducting electric impulses that synchronises chamber contraction, **valves** that allow the generation of high pressure and directional blood flow into the circulation, **coronary circulation**, that supplies oxygenated blood for the heart muscle high metabolically active pumping role and **inlet/outlet routes**, as the venae cavae and pulmonary veins, aorta and pulmonary trunk. This organization highlights the complexity and compartmentalization of the heart. This review will focus on the **cardiac fibroblast**, a cell type until recently ignored, but that profoundly influences heart function in its various compartments. We will discuss current advances on definitions, molecular markers and function of cardiac fibroblasts in heart homeostasis and disease.

Contents

Abbreviations: BCRP1, breakpoint cluster regions pseudogene 1; c-CFU-F, cardiac colony forming unit-fibroblast; CD11b, Integrin, Alpha M (Complement Component 3 Receptor 3 Subunit); CD31, cluster of differentiation 31 or Platelet/Endothelial Cell Adhesion Molecule 1; CD45, cluster of differentiation 45 or Protein Tyrosine Phosphatase, Receptor Type, C Polypeptide; CD90, cluster of differentiation 90 or Thymocyte antigen 1 (Thy-1); CD117, Proto-Oncogene Tyrosine-Protein Kinase Kit or c-Kit; CDC, cardiosphere; CPC, cardiac progenitor cell; Col1a1, Collagen, Type I, Alpha 1; Cx43, connexin 43 or Gap Junction Protein (Gja1), Alpha 1, 43 kDa; Cx45, connexin 45 or Gap Junction Protein (Gja5, Alpha 5, 40 kDa; DDR2, discoidin domain receptor 2; ECM, extracellular matrix; EMT, epithelial to mesenchymal transition; GATA 4/5/6, transcription factor GATA Binding Protein 4/5/6; GFP, green fluorescent protein; Hand2, transcription factor Heart And Neural Crest Derivatives Expressed 2; Klf5, transcription factor Kruppel-Like Factor 5; Mef2c, transcription factor Myocyte Enhancer Factor 2 C; mEF-SK4, anti-feeder antibody, clone mEF-SK4; Nfatc1, transcription factor Nuclear Factor Of Activated T-Cells, Cytoplasmic, Calcineurin-Dependent 1PDGFRa – platelet-derived growth factor receptor alpha; Nkx2–5, transcription factor NK2 Homeobox 5; Pax3, transcription factor Paired Box 3; Sca1, stem cell antigen 1; SMA, smooth muscle actin; Tbx18, T-Box transcription factor 18; Tbx20, T-Box transcription factor 20; Tcf21, Transcription Factor 21; Tie2, Tyrosine Kinase With Ig And EGF Homology Domains-2; Wt1, transcription factor Wilm's tumor factor 1

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1. Introduction

It is estimated that about 45% of all deaths in the developed world involve fibrosis (Wynn, 2008), that is, the exacerbated stimulation of fibroblasts, ultimately resulting in excessive accumulation of extracellular matrix (ECM) components that impair organ function. Nevertheless, until fairly recently, the fibroblast remained neglected and simply considered biological glue, as opposed to an active component of organ physiology. In the heart, cardiac fibroblasts have now gained center stage in regenerative biology, due to their importance for the maintenance of the homeostatic balance, as well as for disease states that result in heart failure.

Unlike fish and other lower organisms that retain cardiomyocyte proliferation capacity through adulthood (Kikuchi, 2014), the mammalian heart shows poor regenerative power due at least in part to terminal differentiation of adult cardiomyocytes (Ahuja et al., 2007; Bergmann et al., 2009; Kajstura et al., 2010; Li et al., 1996). It has been postulated that mammalian organs exchanged plasticity for specialisation, as exemplified by the complex cellular composition and compartmentalization of the heart. However, upon insults that result in extensive cardiomyocyte (or muscle) death, such as myocardial infarction, the organ becomes frail and incapable of coping with the body demands for oxygen and nutrients. Decreased muscle mass needs to somehow be replaced so that the coordinated pump function of the organ is maintained. Since adult cardiomyocytes show low proliferative capacity, this is achieved through fibroblast activation, proliferation and ECM deposition in injured areas, a process named reparative fibrosis (Weber et al., 2013). However, mechanical properties of cardiomyocytes and fibroblast/ECM components are different from the original tissue and lead to increased stiffness and therefore higher workload of the heart, in order to keep up with the body demanding oxygen/nutrient supply. The increased workload prompts a remodelling of the heart, which normally grows in size through cardiomyocyte hypertrophy (increase in cell size) that escalates to dilation, which ultimately impairs heart function to unsustainable levels. This process is called pathological remodelling and leads to heart failure. In addition to myocardial infarction, various cardiovascular conditions leading to heart failure are intimately linked to fibroblast/myofibroblast activity, although not all cardiovascular conditions show extensive muscle death. For example, chronic hypertension leads to diffuse perivascular fibrosis in the heart and cardiomyocyte hypertrophy, without significant cardiomyocyte death (Diez, 2007).

As heart failure has no cure, the only current solution for patients undergoing heart failure is a heart transplant, a very scarce and only palliative solution. According with the American Heart Association, cardiovascular disease accounted for 30.8% (1 in 3) deaths in the United States in 2013, and it is still one of the major burdens to the global health system (Mozaffarian et al., 2015). This highlights the importance of understanding the cardiac fibroblast in full detail, in order to uncover efficient therapeutic solutions for this debilitating and life threatening condition.

2. Heart composition

Previous studies have poised the cardiac fibroblast as the major cell type in the heart, accounting for 30–60% of the total cell number in the heart tissue, although cardiomyocytes occupy a larger volume, due to their cell size and shape (Baudino et al., 2006; Camelliti et al., 2005; Fan et al., 2012; Krenning et al., 2010; Turner, 2011). Conversely, a recently published study demonstrated that the most abundant cell type in the heart is the endothelial cell, which constitutes over 60% of non-myocytes in the heart, while hematopoietic-derived cells constitute 5–10% and fibroblasts 20% of non-myocytes (Pinto et al., 2016). This study used state-of-the-art unsupervised cell clustering analysis, coupled with various genetic tools and cellular markers in the mouse. Similar conclusions were also drawn for the human heart, where populations were counted using immunohistochemistry methods. Although the study still presents limitations, such as biases related to cell death during harsh dissociation procedures and efficacy of markers in picking up the whole population of various cells (endothelial, immune and mesenchymal), it is currently the most systematic study on quantification of heart cell composition.

Cardiac fibroblasts are found in all compartments of the heart, including mural muscle walls and the cardiac skeleton (Fig. 1). The cardiac skeleton is a dense connective tissue structure that separates the atrial from the ventricular compartments, isolating these areas electrically. It also encircles the pulmonary trunk and the aorta, and serves as anchorage for the atrioventricular valves, atrial septum and interventricular septum, all of which are rich in fibroblast composition. Mural fibroblasts are found throughout the muscle compartments, including both atria, as well as ventricles and the interventricular septum. In addition to these compartments, cardiac fibroblasts are also found in the conduction system, where they purportedly form a barrier that insulates electrical impulses, allowing conduction to proceed in a directional fashion (Camelliti et al., 2005; Christoffels and Moorman, 2009).

3. Definition

The field of fibrosis has been largely hampered by nomenclature issues and lack of proper markers that uniformly label the fibroblast pool in homeostasis or disease states. The word fibroblast comes from the Latin '*fibr*' (fiber) and the Greek '*blast*' (germ, cell, bud), referring to the classic definition of the fibroblast as a fiber secreting cell, part of the mesenchymal component of organs. While this definition is accurate, it is not exclusive and causes confusion when trying to isolate and classify a fibroblast. For example, this definition is based on the fact that fibroblasts are capable of producing collagen, among other ECM components. This is not to say that other cell types of the body are incapable of secreting collagen, and therefore may be confused with fibroblasts. Indeed, epithelial (Hayashi et al., 1988; Langness and Udenfriend, 1974), chondrocytes (Muir, 1995) and monocytes/macrophages (Kubin et al., 2011; Li et al., 2011; Peng et al., 2011; Pinto et al., 2012; Rodero et al., 2013; Schnoor et al., 2008; Vaage and Harlos, 1991; Vaage and Lindblad, 1990) are all collagen-producing cells. Some of the commonly used markers for fibroblasts are collagens, filamin A (ECM components), vimentin (intermediate filament), DDR2 (collagen receptor) and Thy-1 or CD90 (GPI-anchored protein of unknown function). None of these markers are either