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ORIGINAL INVESTIGATIONS

Interacting Resident Epicardium-Derived Fibroblasts and Recruited Bone Marrow Cells Form Myocardial Infarction Scar

Adrián Ruiz-Villalba, PhD,*† Ana M. Simón, PhD,‡ Cristina Pogontke, MSc,*§ María I. Castillo, MSc,*§ Gloria Abizanda, MSc,‡ Beatriz Pelacho, PhD,‖ Rebeca Sánchez-Domínguez, PhD,¶# José C. Segovia, PhD,¶# Felipe Prósper, MD, PhD,‡ José M. Pérez-Pomares, PhD*§

ABSTRACT

BACKGROUND Although efforts continue to find new therapies to regenerate infarcted heart tissue, knowledge of the cellular and molecular mechanisms involved remains poor.

OBJECTIVES This study sought to identify the origin of cardiac fibroblasts (CFs) in the infarcted heart to better understand the pathophysiology of ventricular remodeling following myocardial infarction (MI).

METHODS Permanent genetic tracing of epicardium-derived cell (EPDC) and bone marrow–derived blood cell (BMC) lineages was established using Cre/LoxP technology. In vivo gene and protein expression studies, as well as in vitro cell culture assays, were developed to characterize EPDC and BMC interaction and properties.

RESULTS EPDCs, which colonize the cardiac interstitium during embryogenesis, massively differentiate into CFs after MI. This response is disease-specific, because angiotensin II–induced pressure overload does not trigger significant EPDC fibroblastic differentiation. The expansion of epicardial-derived CFs follows BMC infiltration into the infarct site; the number of EPDCs equals that of BMCs 1 week post-infarction. BMC-EPDC interaction leads to cell polarization, packing, massive collagen deposition, and scar formation. Moreover, epicardium-derived CFs display stromal properties with respect to BMCs, contributing to the sustained recruitment of circulating cells to the damaged zone and the cardiac persistence of hematopoietic progenitors/stem cells after MI.

CONCLUSIONS EPDCs, but not BMCs, are the main origin of CFs in the ischemic heart. Adult resident EPDC contribution to the CF compartment is time- and disease-dependent. Our findings are relevant to the understanding of post-MI ventricular remodeling and may contribute to the development of new therapies to treat this disease. (J Am Coll Cardiol 2015;65:2057–66) © 2015 by the American College of Cardiology Foundation.

From the *Department of Animal Biology, Faculty of Sciences, University of Málaga, Málaga, Spain; †Department of Anatomy, Embryology and Physiology, AMC-University of Amsterdam, Amsterdam, the Netherlands; ‡Department of Hematology, Clínica Universitaria de Navarra-CIMA, Universidad de Navarra, Pamplona, Spain; §Andalusian Center for Nanomedicine and Biotechnology, Campanillas (Málaga), Spain; ‖Stem Cell Therapy Area, Foundation for Applied Medical Research, University of Navarra, Pamplona, Spain; ¶Differentiation and Cytometry Unit, Hematopoietic Innovative Therapies Division, Centro de Investigaciones Energéticas, Medioambientales y Tecnológicas-Centro de Investigaciones Biomédicas en Red de Enfermedades Raras, Madrid, Spain; and the #Advanced Therapies Mixed Unit, Instituto de Investigación Sanitaria-Fundación Jiménez Díaz, Madrid, Spain. Dr. Pelacho is supported by ISCIII PI13/02144 and CP09/00333. Dr. Segovia is supported by ISCIII RD12/0019-0023. Dr. Prósper is supported by ISCIII RD12/0019-0032. Dr. Pérez-Pomares is supported by MINECO grant BFU2012-35799, ISCIII RD12/0019-0022, Junta de Andalucía CTS-7564, and EU FP7-Marie Curie-ITN actions PITN-GA-2011-289600. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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ABBREVIATIONS AND ACRONYMS

BMC = bone marrow–derived cell

BZ = border zone

CD = cluster of differentiation

CF = cardiac fibroblast

EPDC = epicardium-derived cell

eYFP = enhanced yellow fluorescent protein

FACS = fluorescence-activated cell sorting

IZ = infarct zone

LT-HSC = long-term hematopoietic stem cell

MI = myocardial infarction

mRFP = monomeric red fluorescent protein

RZ = remote zone

SDF = stromal cell-derived factor

SMA = smooth muscle actin

Affecting millions of people, myocardial infarction (MI) is a leading cause of morbidity and mortality worldwide (1). Given increasing pressure to find clinical alternatives to heart transplantation, which is the only effective treatment for terminal cardiac failure that often results from MI, multiple researchers have sought to find an optimal cellular source to substitute dead or damaged cardiomyocytes with new, functional ones (2). However, knowledge of the cellular and molecular mechanisms that instruct and contribute to the normal, physiological repair of the injured heart remains very limited. This is paradoxical, because any experimental cell addition to the injured heart will eventually interact with endogenous cardiac reparative phenomena. Therefore, understanding such events is necessary to identify an optimal time point to inject cells with therapeutic properties into the damaged organ, and to critically analyze the poor results so far reported by cell infusion–based clinical trials attempting to fix the infarcted heart (3).

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During an MI, cardiac muscle death triggers an acute inflammatory response, led by circulating, bone marrow–derived blood cells (BMCs), which is followed by the mobilization of cardiac fibroblasts (CFs) in the ischemic region (4,5). After an MI, activated CFs expand to repair the injured area and deposit large amounts of extracellular matrix components, mainly collagen (6,7). This excessive deposition soon transforms into a growing scar; this disrupts the kinetic properties of the myocardial walls, impairing the mechanoelectric coupling of cardiomyocytes, increasing the risk of arrhythmias (8,9), and eventually leading to heart failure.

Despite the enormous clinical relevance of CFs, not much is known about their origin and biological properties in the context of ischemic heart disease. Interestingly, it has been suggested that characterizing CF biology in relation to the cells' origin could be instrumental in defining its genetic background and potential response to normal and pathologic conditions (10). Therefore, we aimed to identify the origin of CFs in the infarcted heart and study their interaction with BMCs during fibrotic ventricular remodeling.

METHODS

All animals used in this study were handled in compliance with institutional and European Union guidelines for animal care and welfare under a specific experimental procedure approved by the Ethics Committee of the University of Málaga. Homozygote *Wt1/IRES/GFP-Cre* (*Wt1*Cre) mice ($Cre^{+}/^{+}$) were crossed with *B6.129X1-Gt(ROSA)26Sortm1(EYFP)Cos/J* mice (Rosa26R-eYFP, The Jackson Laboratory, Bay Harbor, Maine). Wilm's Tumor Gene 1 (Wt1)-driven Cre activity mediates the excision of the LoxP-flanked STOP sequence in R26R mice, activating permanent reporter enhanced yellow fluorescent protein ($eYFP^{+}$) expression in the $Wt1^{+}$ cell lineage ($Wt1Cre\text{-}YFP^{+}$). Routine tissue extraction, fixation, and immunohistochemistry were performed as detailed in the Online Appendix (antibodies used are listed in Online Tables 1 and 2).

*Wt1*Cre-$eYFP^{+}$ nonmyocardial cell suspensions were washed in phosphate-buffered saline, and incubated in 2% fetal bovine serum and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid with the proper fluorochrome-conjugated primary immunoglobulin Gs (Online Table 3). Cell suspensions were analyzed in a MoFlo cell sorter (DakoCytomation, Glostrup, Denmark).

For bone marrow transplantations, 12-week to 16-week-old C57/BL and *Wt1*Cre-$eYFP^{+}$ host mice were irradiated and transplanted with bone marrow cells collected from monomeric red fluorescent protein (mRFP) donor mice, allowing for the full reconstitution of the hematopoietic system of recipient mice with constitutive mRFP-expressing cells. One and 3 months after transplantation the hematopoietic engraftment was analyzed by flow cytometry from peripheral blood samples via the analysis of mRFP fluorescence and antibodies against cluster of differentiation (CD) 45.1/CD45.2 pan-leucocyte isoforms (BD Biosciences, San Jose, California).

MI was induced by ligation of the left anterior descending coronary artery of 20- to 24-week-old mice as described elsewhere (11). Routine Mallory trichrome staining was performed to evaluate fibrosis, and samples were processed for immunohistochemistry and eYFP/mRFP visualization as previously mentioned.

Total ribonucleic acid from $eYFP^{+}/CD31^{-}$ cells sorted from dissected sham, remote, and infarcted zones was extracted using Tri-Reagent (Life Technologies, Grand Island, New York) and polymerase chain reaction performed in an Eco thermocycler (Illumina Inc., San Diego, California) as indicated in the Online Appendix.

Stromal cell–derived factor (SDF)-1α (R&D Systems, Minneapolis, Minnesota, 0.1 μg/ml in Hank's Balanced Salt Solution, Life Technologies, Thermo Fisher