International Journal of Cardiology 258 (2018) 217–223

Contents lists available at ScienceDirect

International Journal of Cardiology

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

A role for fumarate hydratase in mediating oxidative effects of galectin-3 in human cardiac fibroblasts

Jaime Ibarrola [a], Rafael Sádaba [a], Amaia Garcia-Peña [a], Vanessa Arrieta [a], Ernesto Martinez-Martinez [a], Virginia Alvarez [a], Amaya Fernández-Celis [a], Alicia Gainza [a], Enrique Santamaría [b], Joaquin Fernández-Irigoyen [b], Victoria Cachofeiro [c], Renaud Fay [d], Patrick Rossignol [d], Natalia López-Andrés [a,d,*]

[a] Cardiovascular Translational Research, Navarrabiomed, Complejo Hospitalario de Navarra (CHN), Universidad Pública de Navarra (UPNA), IdiSNA, Pamplona, Spain
[b] Proteored-ISCIII, Proteomics Unit, Navarrabiomed, Complejo Hospitalario de Navarra (CHN), Universidad Pública de Navarra (UPNA), IdiSNA, Pamplona, Spain
[c] Department of Physiology, School of Medicine, Universidad Complutense, Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Ciber de Enfermedades Cardiovasculares (CIBERCV), Instituto de Salud Carlos III, Madrid, Spain
[d] INSERM, Centre d'Investigations Cliniques-Plurithématique 1433, UMR 1116 Université de Lorraine, CHRU de Nancy, French-Clinical Research Infrastructure Network (F-CRIN) INI-CRCT, Nancy, France

ARTICLE INFO

Article history:
Received 24 October 2017
Received in revised form 15 December 2017
Accepted 28 December 2017

Keywords:
Galectin-3
Fumarate hydratase
Fibroblasts
Aortic stenosis

ABSTRACT

Aims: Galectin-3 (Gal-3), a β-galactoside-binding lectin involved in cardiac inflammation and fibrosis, could regulate oxidative stress, although the mechanisms have not been elucidated. We herein investigated the changes in oxidative stress-related mediators induced by Gal-3 in human cardiac fibroblasts and in pathological animal and human models of cardiac diseases.

Results: Using quantitative proteomics and immunodetection approaches, we have identified that Gal-3 downregulated fumarate hydratase (FH) in human cardiac fibroblasts. In parallel, Gal-3 increased fumarate production in a time-dependent manner. Gal-3 treatment enhanced carbonylated proteins detected through OxyBlot technique. Interestingly, treatment of cells with fumarate induced oxidative stress, enhanced fibroblast activation markers and increased collagen and interleukin-6 secretion. In Gal-3-silenced cells and in heart from Gal-3 knock-out mice, FH was increased and fumarate was decreased. In myocardial biopsies from patients with aortic stenosis (AS, $n = 26$), FH levels were decreased as compared to Controls ($n = 13$). Cardiac Gal-3 inversely correlated with FH levels in myocardial biopsies. In an experimental model of AS rats, pharmacological inhibition of Gal-3 restored cardiac FH, decreased fumarate concentration and improved oxidative status.

Conclusion: In human cardiac fibroblasts, Gal-3 decreased FH expression increasing fumarate concentration and promoting oxidative stress. In human AS, cardiac levels of Gal-3 inversely associated with FH. Gal-3 blockade restored FH and improved fumarate and oxidative stress status in AS rats. FH is therefore a key molecule mediating Gal-3-induced oxidative stress in cardiac cells.

1. Introduction

Galectin-3 (Gal-3) is a member of a β-galactoside binding lectin family that participates in cardiac inflammation, fibrosis and aortic valve calcification, contributing to heart failure (HF) [1,2]. Moreover, Gal-3 has been involved in reactive oxygen species (ROS) production, although the mechanisms have not been elucidated. Gal-3 increases the expression of Nox4 in cardiac cells and could regulate Nox4-derived ROS levels during cardiac fibrosis [3]. Gal-3 could also increase superoxide production in monocytes [4]. Furthermore, Gal-3 positively correlated with markers of oxidative stress (F2-isoprostanes) in peripheral artery disease patients [5], with Nox4 in pulmonary arterial hypertension patients [3] and with NADPH oxidase-dependent superoxide production in asymptomatic subjects with atherothrombosis [6].

Mitochondria are the major source of ROS as well as the major target of ROS damage. Fumarate hydratase (FH) or fumarase is a nuclear-encoded mitochondrial enzyme that takes part in the tri-carboxylic acid cycle, catalyzing the reversible conversion between fumarate and L-malate. Loss of FH has been previously associated with increased production of ROS [7], and the accumulation of fumarate enhances oxidative stress [8]. Thus, accumulation of fumarate caused by the inactivation of FH leads to oxidative stress [9]. In line with these observations, fumarate excess exacerbated salt-induced hypertension and cellular levels of hydrogen peroxide in a hypertensive model [10]. Recently, it has been described that Nox4 potently inhibited FH, leading to

* Corresponding author at: Cardiovascular Translational Research, Navarrabiomed (Miguel Servet Foundation), C/Irunlarrea 3, 31008 Pamplona, Spain.
E-mail address: natalia.lopez.andres@navarra.es (N. López-Andrés).

https://doi.org/10.1016/j.ijcard.2017.12.103
0167-5273/

accumulation of fumarate in kidney and contributing to renal fibrosis and pathology [11].

In the present study, a proteomic approach has been used for the characterization of the proteostasis impairment after Gal-3 treatment. The possible effects of Gal-3 on FH in adult cardiac fibroblasts have been investigated. Then, results have been strengthened in myocardial biopsies from aortic stenosis (AS) patients. Finally, the impact of Gal-3 genetic disruption and pharmacological inhibition on FH in experimental models has been analyzed.

2. Methods

Detailed methods are available in the supplemental material.

2.1. Cell culture

Human Cardiac Fibroblasts were obtained from Promocell and stimulated with Gal-3 (10^{-8} M, R&D Systems) [1] and fumarate (25–100 mM, Santa Cruz) for 72 h for protein analysis and for 6 h for mRNA analysis.

2.2. Mass spectrometry based-quantitative proteomics

A shotgun comparative proteomic analysis of untreated cardiac fibroblasts and cardiac fibroblasts stimulated with Gal-3 was performed using iTRAQ [12,13].

2.3. In vivo studies

Adult male Wistar rats obtained from Harlan Ibérica were distributed in three groups; Control ($n = 7$), rats subjected to supravalvular aortic banding mimicking AS (AS; n = 7) and AS rats receiving the Gal-3 inhibitor (AS + MCP; 100 mg/kg/day; n = 7) [14–16].

Adult male C57BJ6 WT mice and Gal-3 knock-out (KO) mice were used [17,18].

The Animal Care and Use Committee of Universidad Complutense de Madrid approved all experimental procedures according to guidelines for ethical care of experimental animals of the European Community.

2.4. Myocardial biopsies

Myocardial biopsies were obtained from patients with severe AS ($n = 26$), referred to our center for aortic valve replacement. As controls, myocardial biopsies from subjects who have died from non-cardiovascular-related diseases were obtained at autopsy (Control, $n = 13$). Informed consent was obtained from each patient and control and the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee [14].

2.5. Statistical analyses

For human studies, continuous variables were expressed as mean ± SD or median (25th to 75th percentile) and compared using unpaired T student test. Spearman's correlation coefficients were calculated to determine correlations. For cellular and animal studies, data are expressed as mean ± SEM. Data were analyzed using a one-way analysis of variance, followed by a Newman-Keuls to assess specific differences among groups or conditions using GraphPad Software Inc. The predetermined significance level was $p < 0.05$.

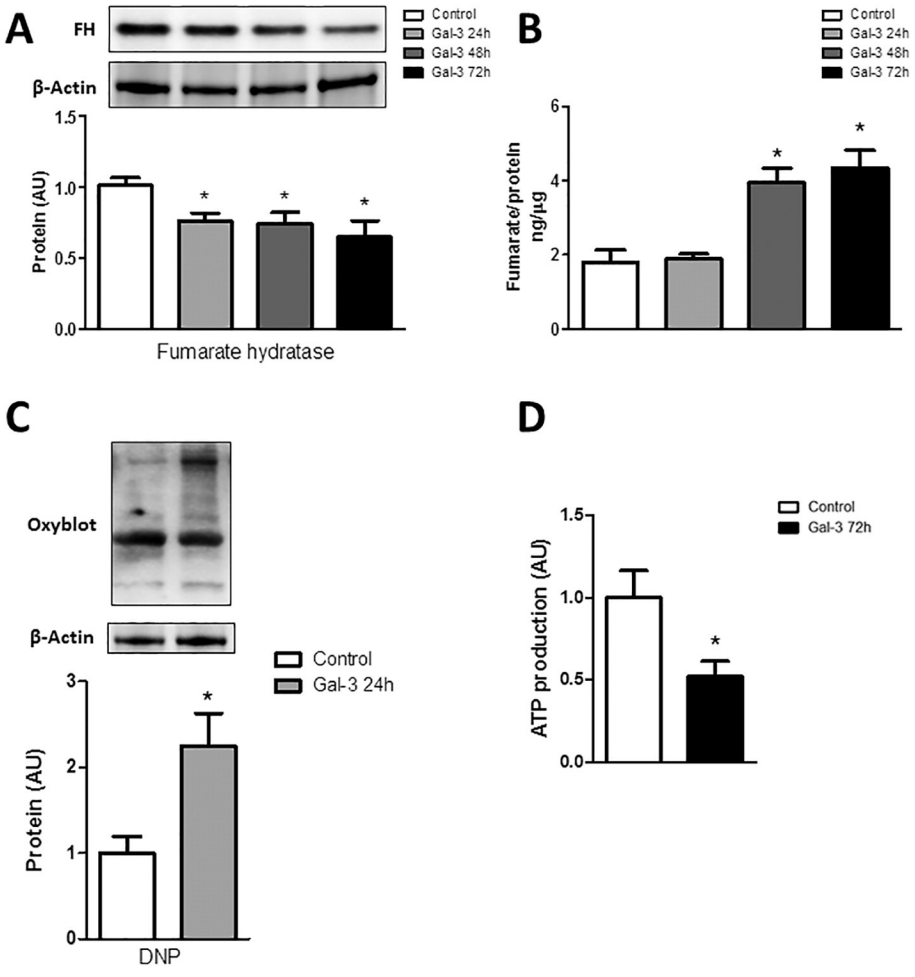

Fig. 1. FH expression in adult human cardiac fibroblasts treated with Gal-3. FH protein expression (A) and fumarate production (B) in adult human cardiac fibroblasts treated with Gal-3 (10^{-8} M) for 24, 48 and 72 h. Protein carbonyl quantification in cells treated with Gal-3 (10^{-8} M) for 24 h (C). ATP production in cells treated with Gal-3 (10^{-8} M) for 72 h (D). All conditions were performed at least in triplicate. Histogram bars represent the mean ± SEM of 6 assays in arbitrary units (AU) normalized to stain free and β-actin for protein. *$p < 0.05$ vs Control. Gal-3, Galectin-3; DNP, dinitrophenyl hydrazone product; FH: fumarate hydratase.