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Original article

Intermittent hypoxia-induced cardiomyopathy and its prevention by Nrf2 and metallothionein

Shanshan Zhou[a,b], Xia Yin[a], Jingpeng Jin[c], Yi Tan[b,d], Daniel J. Conklin[e], Ying Xin[f], Zhiguo Zhang[a,b], Weixia Sun[a,b], Taixing Cui[g], Jun Cai[b], Yang Zheng[a,*], Lu Cai[b,d,e,**]

[a] The Center of Cardiovascular Diseases, the First Hospital of Jilin University, Changchun 130021, China
[b] Pediatric Research Institute, the Department of Pediatrics of University of Louisville, Louisville 40202, USA
[c] Endoscopy Center China-Japan Union Hospital of Jilin University, 126 Xiantai Street, Changchun 130033, China
[d] Chinese-American Research Institute for Diabetic Complication, Wenzhou Medical College, Wenzhou 325035, China
[e] Diabetes and Obesity Center, University of Louisville, Louisville, KY 40202, USA
[f] Key Laboratory of Pathobiology, Ministry of Education, Jilin University, Changchun 130021, China
[g] Department of Cell Biology and Anatomy, University of South Carolina, School of Medicine, Columbia, SC 29208, USA

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ABSTRACT

The mechanism for intermittent hypoxia (IH)-induced cardiomyopathy remains obscure. We reported the prevention of acute and chronic IH-induced cardiac damage by selective cardiac overexpression of metallothionein (MT). Herein we defined that MT-mediated protection from IH-cardiomyopathy is via activation of nuclear factor erythroid 2-related factor 2 (Nrf2), a critical redox-balance controller in the body. For this, mice were exposed to IH for 3 days (acute) or 4 or 8 weeks (chronic). Cardiac Nrf2 and MT expression in response to IH were significantly increased acutely yet decreased chronically. Interestingly, cardiac overexpression (Nrf2-TG) or global deletion of the *Nrf2* gene (Nrf2-KO) made mice highly resistant or highly susceptible, respectively, to IH-induced cardiomyopathy and MT expression. Mechanistically, 4-week IH exposure significantly decreased cardiac Nrf2 binding to the MT gene promoter, and thus, depressed both MT transcription and translation in WT mice but not Nrf2-TG mice. Likewise, cardiac MT overexpression prevented chronic IH-induced cardiomyopathy and down-regulation of Nrf2 likely via activation of a PI3K/Akt/GSK-3β/Fyn-dependent signaling pathway. These results reveal an integrated relationship between cardiac Nrf2 and MT expression in response to IH – acute compensatory up-regulation followed by chronic down-regulation and cardiomyopathy. Cardiac overexpression of either Nrf2 or MT offered cardioprotection from IH via complicated PI3K/Akt/GSK3B/Fyn signaling. Potential therapeutics may target either Nrf2 or MT to prevent chronic IH-induced cardiomyopathy.

1. Introduction

Intermittent hypoxia (IH) has received considerable attention in clinical settings in recent years increasing awareness of its biological and clinical significance. A key reason for extensive interest in IH is its occurrence in sleep-disordered breathing such as obstructive sleep apnea (OSA) syndrome, as well as in a variety of other diseases, including anomalies of the upper airway (anatomical, functional, or both), obesity, age (> 60 years), smoking, alcohol consumption, somnolence, cognitive impairment, and cardiovascular morbidity and mortality [1–4].

OSA is a well-known public-health problem because of its high prevalence and severe effects. Even in the presence of confounding factors, such as age, sex, obesity, and dyslipidemia, evidence for associations between OSA, cardiovascular diseases and cardiovascular mortality is accumulating [5–9]. As one of the pathogenic factors caused by OSA, intermittent hypoxia (IH) plays a pivotal role in cardiovascular disease [10]. Chronic exposures to IH cause hypertension

List of Abbreviations: OSA, obstructive sleep apnea; IH, intermittent hypoxia; ROS, reactive oxygen; RNS, nitrogen species; MT, Metallothionein; Nrf2, Nuclear factor erythroid 2-related factor 2; AREs, antioxidant response elements; Keap1, kelch-like ECH-associated protein 1; SFN, Sulforaphane; Nrf2-KO, global deletion of Nrf2 gene; MT-KO, global deletion of MT gene; Nrf2-TG, cardiomyocyte-specific overexpression of Nrf2; MT-TG, cardiomyocyte-specific overexpression of MT; LVID,d, LV internal dimension in diastole; LVID,s, LV internal dimension in systole; LVEF, LV ejection fraction; LVFS, LV fractional shortening; IVS, d, interventricle septum thickness in diastole; IVS, s, interventricle septum thickness in systole; LVPW,d, LV posterior wall thickness in diastole; LVPW,s, LV posterior wall thickness in systole

* Corresponding author.
** Correspondence to: Pediatric Research Institute, University of Louisville, 570 S. Preston Street, Baxter I, Suite 304F, Louisville, KY 40202, USA.
E-mail addresses: zhengyang@jlu.edu.cn (Y. Zheng), l0cai001@louisville.edu (L. Cai).

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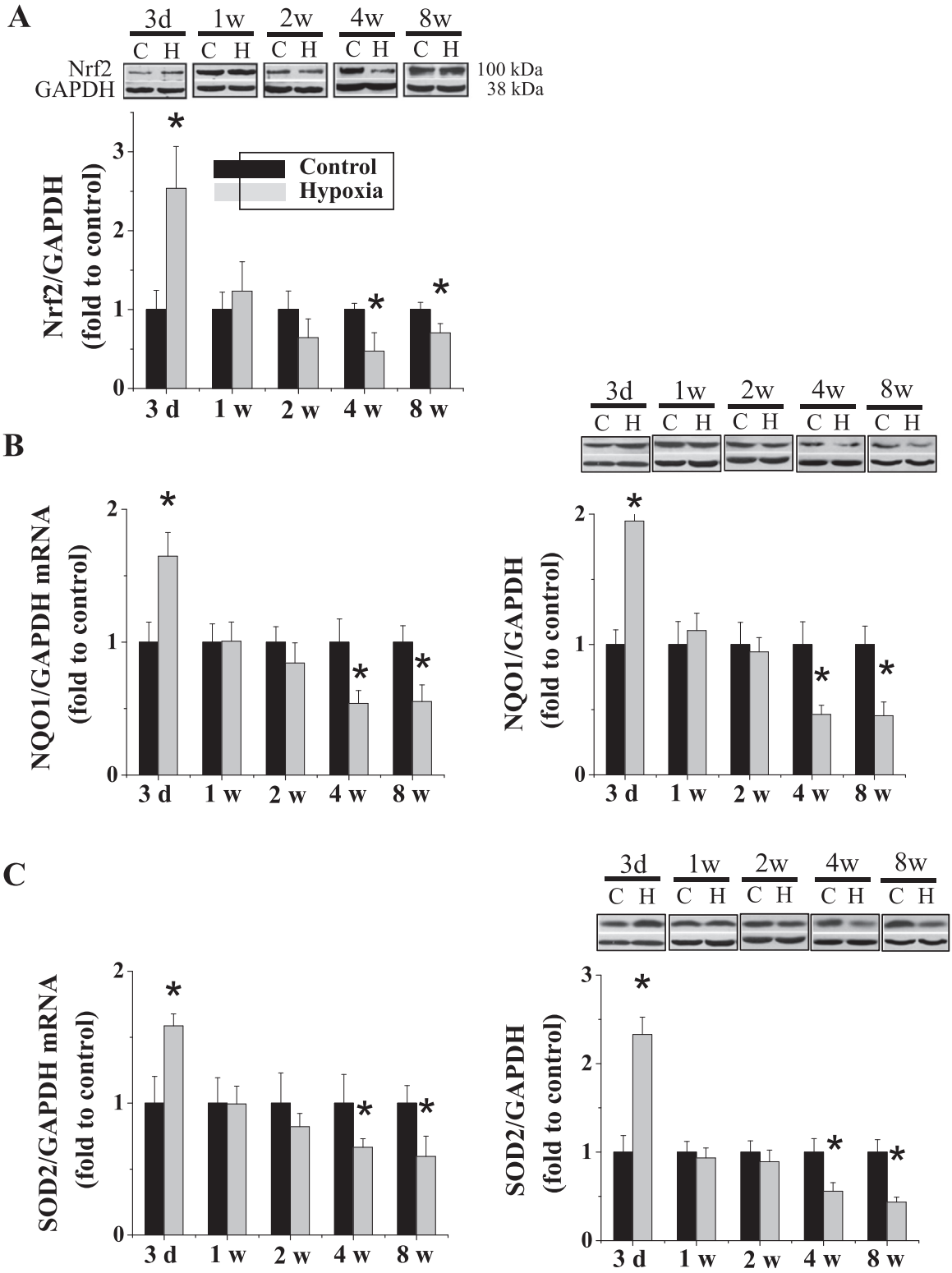

Fig. 1. Expression of Nrf2 in response to IH exposures. FVB WT mice were exposed to IH for indicated times. Cardiac Nrf2 (A) expression was measured by Western blots with its antibody from Abcam (ab137550) with molecular weight of ~ 95 – 110 as suggested by Lau et al. [60]. The NQO1 (B) and SOD2 (C) mRNA was measured by RT-PCR and Western blots. Data are presented as mean ± SD (n = 5). *, $p < 0.05$ vs control.

and cardiac abnormalities in both OSA patients and rodent models of IH [11,12] including left ventricular (LV) remodeling and dysfunction [13,14].

Based on extensive animal studies, oxidative stress is linked to IH-induced cardiac damage [15–18]. Oxidative stress represents an imbalance between the production of reactive oxygen and/or nitrogen species (ROS and/or RNS) and the antioxidant capacity of a biological system to buffer ROS and/or RNS [16]. Metallothioneins (MT) are a family of cysteine-rich, low molecular weight proteins that have binding capacity for physiological and xenobiotic heavy metals through cysteine thiol groups [19]. Recently we demonstrated that endogenous MT expression was up-regulated in response to acute IH, but