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Original article

Experimental heart failure modelled by the cardiomyocyte-specific loss of an epigenome modifier, DNMT3B☆

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ABSTRACT

Differential DNA methylation exists in the epigenome of end-stage failing human hearts but whether it contributes to disease progression is presently unknown. Here, we report that cardiac specific deletion of *Dnmt3b*, the predominant DNA methyltransferase in adult mouse hearts, leads to an accelerated progression to severe systolic insufficiency and myocardial thinning without a preceding hypertrophic response. This was accompanied by widespread myocardial interstitial fibrosis and myo-sarcomeric disarray. By targeted candidate gene quantitative RT-PCR, we discovered an over-activity of cryptic splice sites in the sarcomeric gene *Myh7*, resulting in a transcript with 8 exons missing. Moreover, a region of differential methylation overlies the splice site locus in the hearts of the cardiac-specific conditional knockout (CKO) mice. Although abundant and complex forms of alternative splice variants have been reported in diseased hearts and the contribution of each remains to be understood in further detail, our results demonstrate for the first time that a link may exist between alternative splicing and the cardiac epigenome. In particular, this gives the novel evidence whereby the loss of an epigenome modifier promotes the development and progression of heart disease.

1. Introduction

Despite remarkable improvements in medical therapy, the prognosis of patients with cardiac failure remains poor, with almost 50% of patients dying within 5 year of initial diagnosis. The incidence and prevalence of cardiac failure is on the rise rapidly worldwide [1]. Thus, new therapeutic approaches are urgently needed. Cardiac failure develops often after a prolonged asymptomatic phase, accompanied by changes in heart mass, size and shape, a process known as pathological remodelling [2,3]. It is attributed in some cases to a complex genetic predisposition and/or multiple environmental factors [4]. Nonetheless, gene expression changes are often consistent in a failing heart, regardless of the original inciting cause. Therefore, it would be important to explore whether regulating gene expression in heart failure could be a therapeutic approach. One mechanism of gene expression regulation that has gained importance is epigenetics. The epigenome, unlike the genome, undergoes dynamic changes throughout the course of life. The epigenome, modifiable by diet and environment, may hence contribute to and maintain adaptive and deviant gene expression states [5,6]. Globally, the patterns of DNA methylation established by de novo DNA methyltransferases DNMT3a and DNMT3b during embryogenesis are maintained by DNMT1 and remain stable throughout development and adulthood. However, age, sex and environmental cardiovascular risk factors have been associated with specific alteration of DNA methylation at individual loci [7–9]. Genome-wide profiling of DNA methylation in blood from participants in the Normative Aging study showed that lower LINE-1 methylation in peripheral blood leukocytes is a predictor of incidence and mortality from ischemic heart disease and stroke [10]. Experimental animal models have demonstrated that DNA methylation plays a critical role in the development of atherosclerosis and cardiovascular disease [8]. In humans, *DNMT3B* mutations are causing the rare autosomal recessive disorder *I*mmunodeficiency, *C*entromeric instability and *F*acial anomalies (ICF) Syndrome, associated with severe mental retardation disorders and immunodeficiency [11]. Characterization of the heart has not

☆ One sentence summary: Loss of DNA methyltransferase 3B leads to progressive heart failure.

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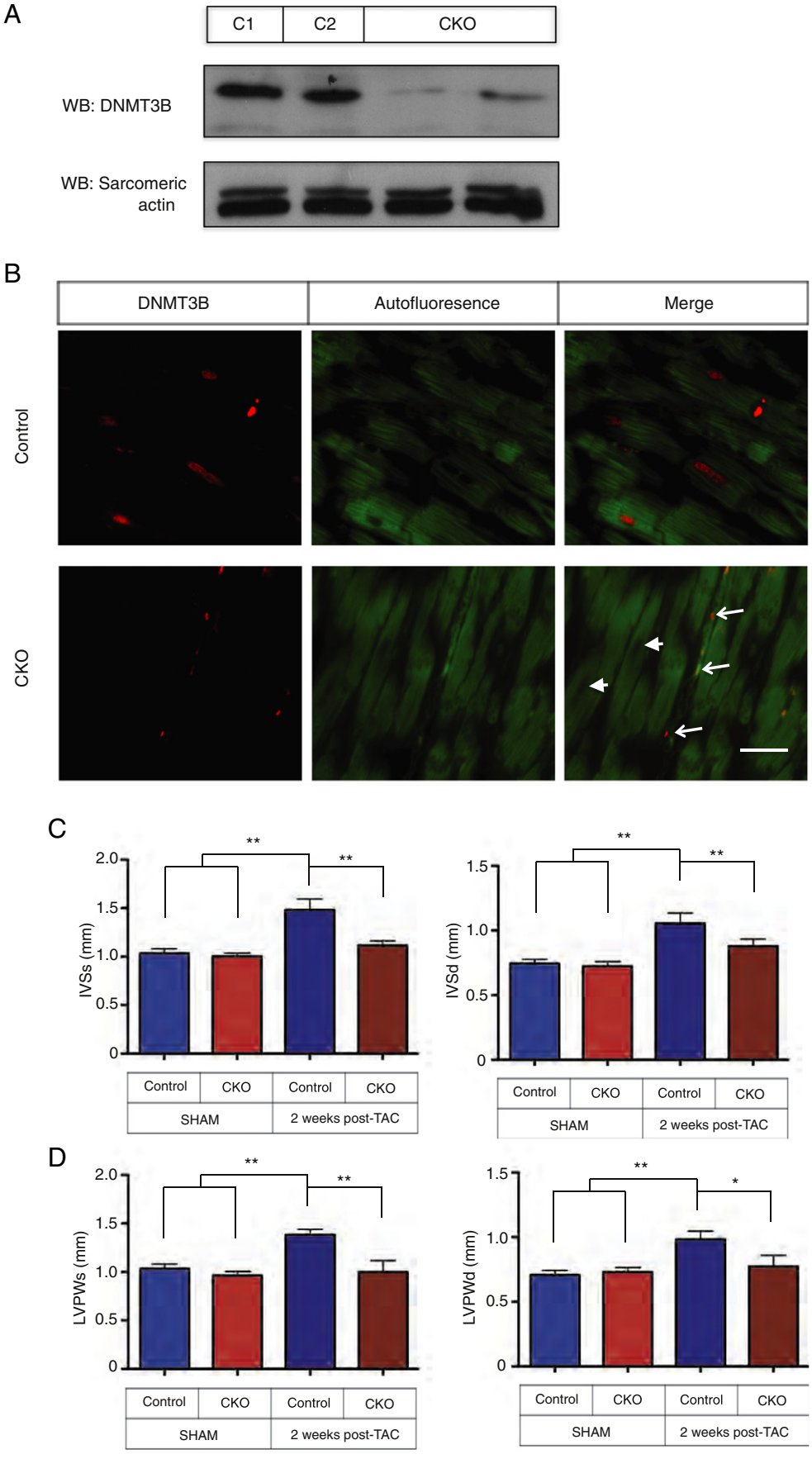
A
C1
C2
CKO
WB: DNMT3B
WB: Sarcomeric actin
B
DNMT3B
Autofluoresence
Merge
Control
CKO
C
IVSs (mm)
IVSd (mm)
2.0
1.5
1.0
0.5
0
**
**
Control
CKO
Control
CKO
SHAM
2 weeks post-TAC
D
LVPWs (mm)
LVPWd (mm)
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