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FAT10 protects cardiac myocytes against apoptosis

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ABSTRACT

FAT10 is a new member of the ubiquitin-like protein family with yet-to-be defined biological functions in the heart. Our objective was to determine the role of FAT10 in the heart. FAT10 is expressed in the normal human and murine hearts, as detected by qPCR and Western blotting. Expression of FAT10 is increased in the heart at the border zone of myocardial infarction and in cultured neonatal rat cardiac myocytes (NRCM) subjected to hypoxia/reoxygenation (H/R) stress. Lentiviral-mediated overexpression of FAT10 in NRCM reduced p53 (TP53) and its target miR-34a levels, while BCL2 level, a target of miR-34a, was increased and BAX level, a pro-apoptotic protein, was reduced. These changes were associated with reduced apoptosis, detected by FACS analysis of annexin-V expression and TUNEL assay, in response to H/R injury. Knock down of FAT10 by shRNA targeting had the opposite effects. Likewise, lentiviral mediated expression of miR-34a was associated with reduced BCL2 and increased BAX levels in NRCM and also reversed changes in BCL-2 and BAX levels observed upon over-expression of FAT10. Treatment of NRCM with proteasome inhibitor MG132 increased p53 and miR-34a levels and reduced BLC2/BAX ratio. These changes were not reversed upon over-expression of FAT10. Thus, FAT10 is upregulated in the heart and NRCM in response to H/R stress, which protects cardiac myocytes against apoptosis. The anti-apoptotic effects of FAT10 are associated with suppression of p53, probably through fatylation and proteasomal degradation, reduced miR-34a expression, and a shift in the BCL2/BAX proteins against apoptosis. Thus, FAT10 is a cardioprotective protein.

1. Introduction

The ubiquitin–proteasome system (UPS) regulates degradation of most cellular proteins [1]. UPS is a key regulator of cardiac function under both physiological and pathological conditions [2–4]. Impaired cardiac UPS functions have been demonstrated in a variety of cardiac pathological states including myocardial ischemia, cardiomyopathies and heart failure [4–6]. Recently several ubiquitin-like proteins with structural and functional similarities to ubiquitin, such as SUMO and NEDD8 have been identified and implicated in targeting protein substrates for possible degradation [7–9].

FAT10 encodes FAT10 (human leukocyte antigen F-associated transcript 10); also known as ubiquitin D (UbD), which is an enigmatic ubiquitin-like protein modifier with poorly defined biological functions [10,11]. It contains two tandem ubiquitin-like (UBL) domains with 30% sequence identity to ubiquitin that might form covalent conjugates with its substrates and target them for 26S proteasomal degradation [12,13]. Expression of FAT10 is induced in response to inflammatory cytokines, such as TNF-α and suppressed by tumor suppressor protein p53 (TP53) [14,15]. The role of FAT10 in apoptosis is unsettled. FAT10-deficient mice are prone to spontaneous apoptosis of lymphocytes [16]. Over-expression of FAT10 in cultured cells, however, is associated with apoptosis [12].

Expression and potential biological functions of FAT10 in the heart are unknown. We show that FAT10 is expressed in cardiac myocytes and its expression protects the myocytes against apoptosis through suppression of p53 and miR-34a and increased expression of BCL2. These findings render FAT10 as a novel cardioprotective gene that is upregulated in response to hypoxic/ischemic injury.

2. Materials and methods

2.1. Animals and surgical procedures

All animal studies were approved by the Animal Ethics and Experimentation Committee of Nanchang University, and performed in

Abbreviations: NRCM, neonatal rat cardiac myocytes; H/R, hypoxia/reoxygenation; UPS, ubiquitin–proteasome system; MI, Myocardial infarction; LAD, left anterior descending artery; FITC, fluorescein isothiocyanate; Co-IP, co-immunoprecipitation; Lt-FAT10, lentivirus containing *Fat10* gene.

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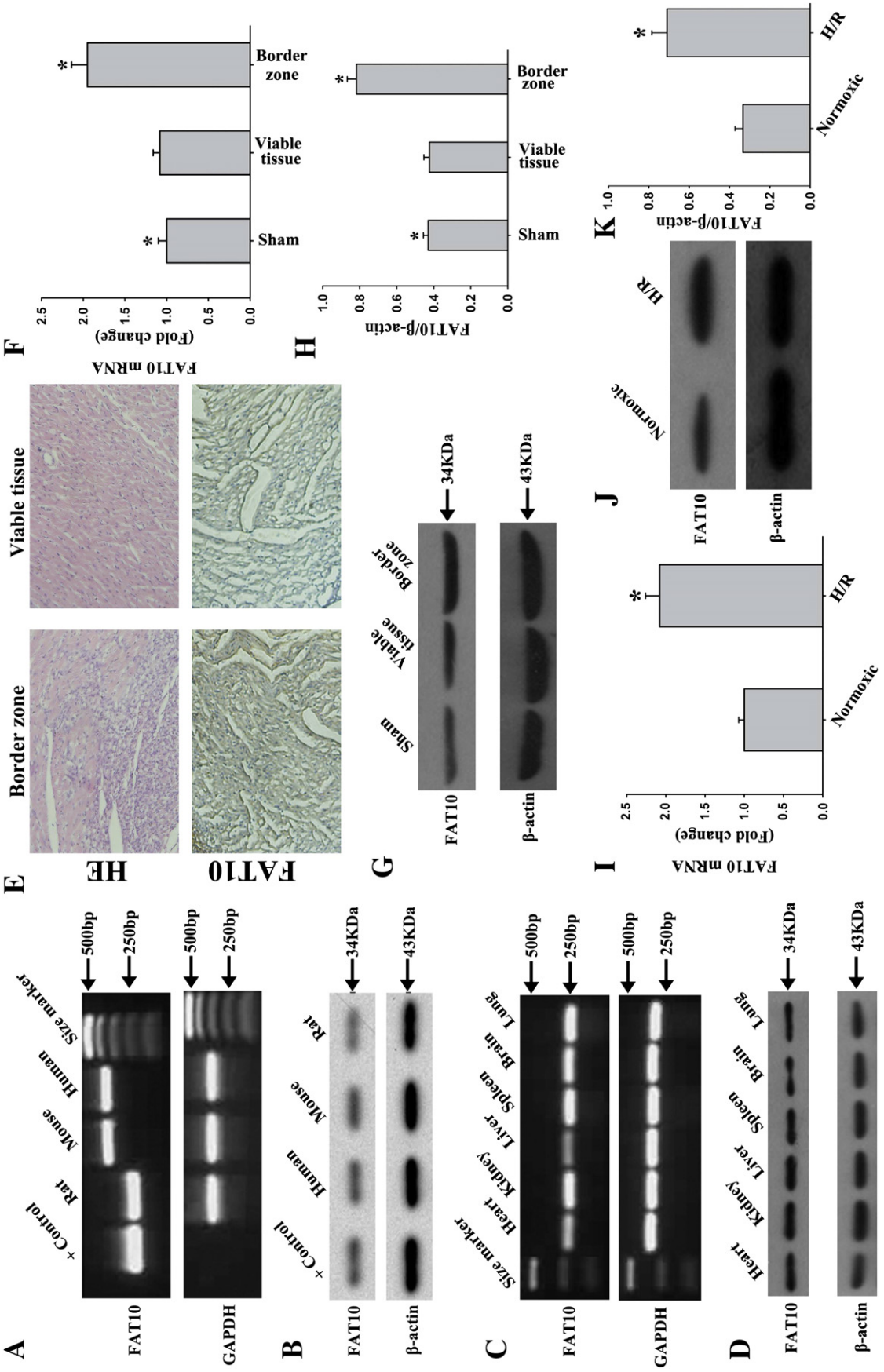
A
+Control
Rat
Mouse
Human
Size marker
500bp
250bp
FAT10
GAPDH
B
+Control
Human
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34KDa
43KDa
FAT10
β-actin
C
Size marker
Heart
Kidney
Liver
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Brain
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