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Q1 Calpain-1 induces endoplasmic reticulum stress in promoting cardiomyocyte apoptosis following hypoxia/reoxygenation

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ABSTRACT

Both calpain activation and endoplasmic reticulum (ER) stress are implicated in ischemic heart injury. However, the role of calpain in ER stress remains largely elusive. This study investigated whether calpain activation causes ER stress, thereby mediating cardiomyocyte apoptosis in an in vitro model of hypoxia/re-oxygenation (H/R). In neonatal mouse cardiomyocytes and rat cardiomyocyte-like H9c2 cells, up-regulation of calpain-1 sufficiently induced ER stress, JNK1/2 activation and apoptosis. Inhibition of ER stress or JNK1/2 prevented apoptosis induced Q6 by calpain-1. In an in vitro model of H/R-induced injury in cardiomyocytes, H/R was induced by a 24-hour hypoxia followed by a 24-hour re-oxygenation. H/R activated calpain-1, induced ER stress and JNK1/2 activation, and triggered apoptosis. Inhibition of calpain and ER stress blocked JNK1/2 activation and prevented H/R-induced apoptosis. Furthermore, blockade of JNK1/2 signaling inhibited apoptosis following H/R. The role of calpain in ER stress was also demonstrated in an in vivo model of ischemia/reperfusion using transgenic mice over-expressing calpastatin. In summary, calpain-1 induces ER stress and JNK1/2 activation, thereby mediating apoptosis in cardiomyocytes. Accordingly, inhibition of calpain prevents ER stress, JNK1/2 activation and apoptosis in H/R-induced cardiomyocytes. Thus, ER stress/JNK1/2 activation may represent an important mechanism linking calpain-1 to ischemic injury.

1. Introduction

Ischemic heart disease is the leading cause of death in most industrialized nations as it causes serious complications such as myocardial infarction (MI). After MI, ischemia-induced myocardial cell death is followed by a progressive remodeling of the heart [1–3], resulting in a severe state of compromised heart function known as heart failure. Heart failure is on the rise as a result of successes in treating acute MI, and up to 40–50% of people with heart failure die within five years of diagnosis [4]. Thus, limiting ischemic injury is extremely important in the prevention of adverse myocardial remodeling and the progression to heart failure.

Calpains are a family of calcium-dependent thiol-proteases [5]. In mammals, fifteen gene products of the calpain family have been reported. Among them, calpain-1 (μ-form) and calpain-2 (m-form) are ubiquitously expressed, and most extensively studied. Calpain-1 and calpain-2 are heterodimers, consisting of a distinct large 80-kDa catalytic subunit encoded by the genes *capn1* and *capn2*, respectively, and a common small 28-kDa regulatory subunit encoded by *capn4*. They differ in their calcium requirements for activation (~50 μM for calpain-1 and ~1000 μM for calpain-2). Both calpain-1 and calpain-2 are tightly regulated by calpastatin, an endogenous inhibitor that specifically inhibits calpain, but not other cysteine proteases. Over-expression of calpastatin has been widely used to inhibit calpain in a variety of in vitro [6] and in vivo models [7,8]. Calpains participate in cardiac patho-physiology. In cultured cardiomyocytes, we and others demonstrated that calpain-1 is important in promoting cardiomyocyte apoptosis under various pathological conditions [9–11]. In response to hypoxia, calpain is activated and contributes to cardiomyocyte injury [12]. In animal models of ischemia/reperfusion or MI, both pharmacologic and genetic inhibitions of calpain reduce ischemic cardiac injury, attenuate myocardial

Abbreviations: ER, endoplasmic reticulum; H/R, hypoxia/re-oxygenation; I/R, ischemia/reperfusion; JNK1/2, c-Jun N-terminal protein kinase1/2; MI, myocardial infarction; MOI, multiplicity of infection; TAUR, tauroursodeoxycholate; CI-III, calpain inhibitor-III; CAST, calpastatin; PERK, protein kinase-like ER kinase; IRE1, inositol-requiring kinase 1; ATF6, activating transcription factor 6; eIF2α, eukaryotic translation initiation factor 2α; ATF4, translation of transcription factor ATF4; XBP1, X-box binding protein 1; CHOP, the transcription factor C/EBP homologous protein; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase

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remodeling and improve myocardial function [13–17]. Furthermore, transgenic over-expression of calpain-1 is sufficient to induce dilated cardiomyopathy and heart failure [7]. These previous studies support an important role of calpain in ischemic heart disease [18]. However, the mechanisms relating calpain activation to ischemic myocardial injury have not been fully addressed.

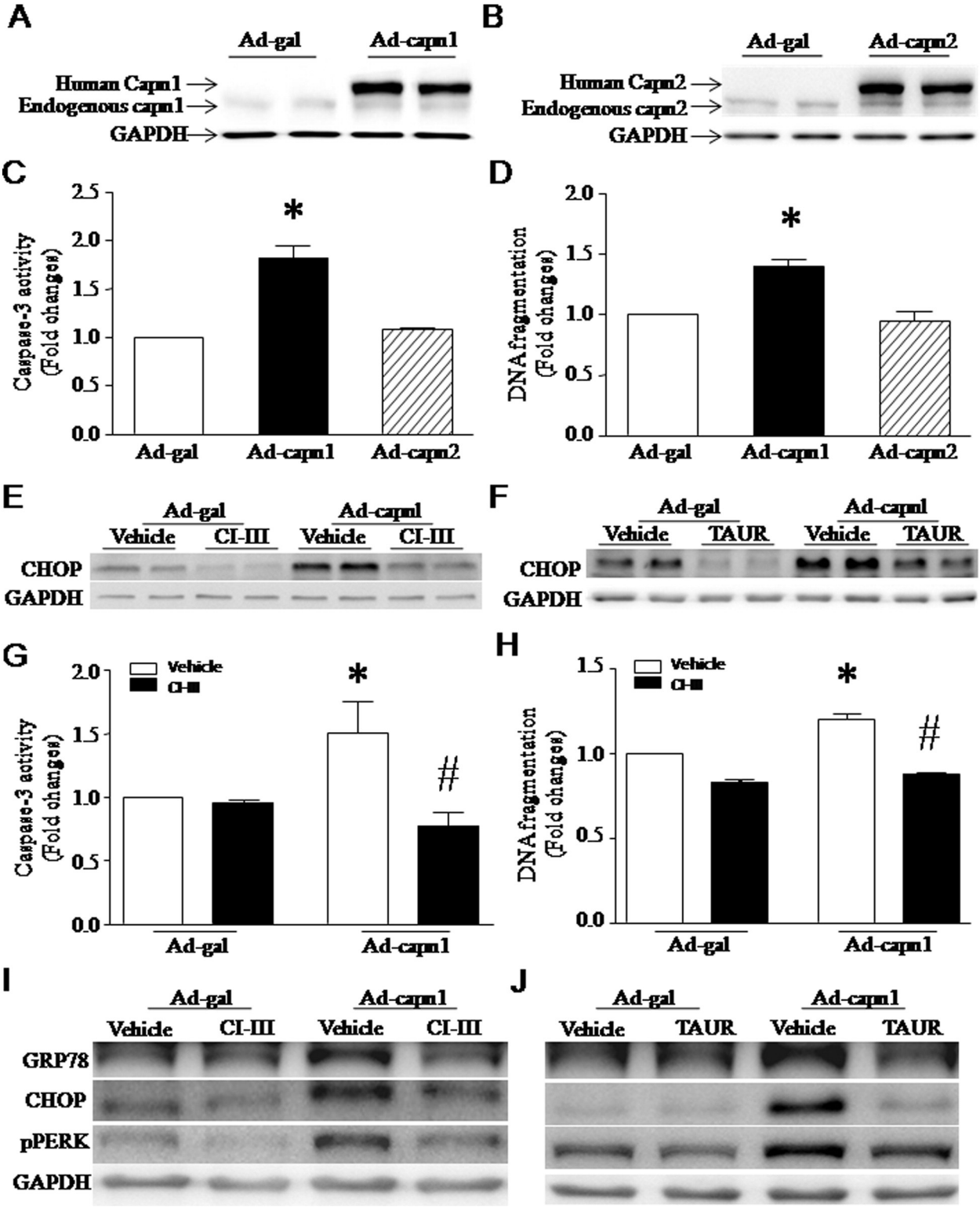

Fig. 1. Apoptosis and ER stress induced by infection with Ad-capn1. (A–F) Cultured neonatal mouse cardiomyocytes were infected with Ad-capn1, Ad-capn2 or Ad-gal as a control, and then incubated with calpain inhibitor-III (CI-III), TAUR or vehicle. Twenty-four hours later, western blot was performed to analyze capn1, capn2, CHOP and GAPDH proteins, and apoptosis was assessed by measuring caspase-3 activity and DNA fragmentation. (A) A representative western blot for capn1 protein from 3 different experiments. (B) A representative western blot for capn2 protein from 3 different experiments. (C) Caspase-3 activity. (D) DNA fragmentation. (E and F) Representative western blots from 3 different experiments for CHOP and GAPDH proteins. (G and H) H9c2 cells were infected with Ad-capn1 or Ad-gal as a control, and then incubated with CI-III, TAUR or vehicle. Twenty-four hours later, apoptosis was assessed by caspase-3 activity (G) and DNA fragmentation (H). (I and J) Representative western blots from 3 different experiments for GRP78, CHOP, pPERK and GAPDH. Data are mean ± SD from 3 different experiments. $^{*}P < 0.05$ vs Ad-gal or Ad-gal + Vehicle and $^{\#}P < 0.05$ vs Ad-capn1 + Vehicle.

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