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Cathepsin B Induced Cardiomyocyte Hypertrophy Requires Activation of the Na^+/H^+ Exchanger Isoform–1

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Background

Progression of the heart to failure is primarily caused due to significant remodeling of both the extracellular matrix (ECM) and subcellular organelles, a hallmark of cardiac hypertrophy (CH). Uncontrolled ECM remodeling occurs as a result of the activation and increased proteolytic activities of proteases such as Cathepsin B (Cat B) and matrix metalloproteinase–9 (MMP-9) (1, 2). Previous studies have suggested that the activation of Cat B is induced by the acidification of the peri and extracellular space (3–5). In various forms of carcinomas, this pericellular acidification coincides with the activation of the cardiac specific pH regulator, the Na^+/H^+ exchanger isoform–1 (NHE1) (5, 6). Increased activation of NHE1, similar to Cat B, is involved in the pathogenesis of various cardiac diseases including CH (7–10). Moreover, the activation NHE1 has been shown to activate Cat B in various reports. CD44 was shown to interact with NHE1 which created an acidic microenvironments leading to Cat B activation in a breast cancer model (5). Moreover, NHE1 and Cat B have shown to directly interact with each other and cause ECM degradation in another breast cancer model (4). Taken together, the evidence suggests that NHE1, through its pH regulating property, might be mediating the activity of Cat B in pathological states. A previous report has demonstrated that pericellular acidification redistributed the Cat B containing lysosomes to the cell surface and caused the secretion of Cat B into the extracellular compartment (3). Interestingly, the NHEs have also shown to cause acidic extracellular pH which induced lysosome trafficking and subsequent release of Cat B into the ECM in a prostate cancer cells (11). Moreover, several broad and specific NHE inhibitors were able to inhibit this effect (11). Once into the extracellular compartment, Cat B can degrade the ECM (12) and facilitate further ECM degradation by activating other proteases such as MMP-9 (13, 14). MMP-9 activity has been shown to be increased in various models of heart failure (15, 16) (17, 18). Previous studies have also shown that MMP-9 activity was increased in CCL39 cells upon the stimulation

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of NHE1 with phenylephrine (19). Interestingly, Cat B and MMP–9 were shown to directly interact with NHE1 and cause ECM degradation in breast cancer (4). Whether NHE1 induces the activation of Cat B, which in turn activates MMP-9 and contributes to cardiomyocyte hypertrophy remains unclear.

Methods

H9c2 cardiomyocytes were treated with 10 μM Angiotensin (Ang) II for 24 hours to stimulate NHE1 and to induce cardiomyocyte hypertrophy. Cells were further treated with or without 10 μM EMD, a NHE1 inhibitor, or 10 μM CA-074 methyl ester (CA-074Me), a Cat B inhibitor, for 24 hours. After treatments, Cat B messenger ribonucleic acid (mRNA) levels were measured through Reverse Transcription-Polymerase Chain Reaction (RT-PCR). Furthermore, changes in the cardiomyocyte hypertrophic marker, ANP mRNA, were also assessed by RT-PCR analysis. The localization of Cat B in lysosomes was measured using LysoTracker Red dye. Autophagy was measured through the analysis of the autophagic marker, microtubule associated light chain 3–II (LC3–II). The secretion of Cat B from the intracellular to the extracellular space was assessed by measuring Cat B protein expression in the media. MMP–9 activity was also measured in the media by gelatin zymography and assessed for its contribution to the Cat B hypertrophic response.

Results

Immunoblot analysis revealed that Cat B protein expression, both pro and active forms, was significantly elevated at the 10 μM Ang II concentration (136.56 $\pm$ 9.4% Ang II vs. 100% control, 37 kDa and 169.84 $\pm$ 14.24% Ang II vs. 100% control, 25 kDa; P