GLOBAL SCIENCE LIBRARY

RESEARCH

Journal of Cardiology Research

Dysregulation of H11 Kinase in the Failing Left Ventricular Myocardium

Ramesh C Gupta*, Vinita Singh-Gupta and Hani N Sabbah

Department of Medicine, Division of Cardiovascular Medicine, Henry Ford Hospital, USA

Abstract

Background: *Increased expression of H11 kinase (H11K) has been observed in Left Ventricular (LV) myocardium of failed human hearts. Its potential role as a contributor to the progression of Heart Failure (HF) remains uncertain. In the present study we examined the expression of H11K in cytosolic and mitochondrial fractions of failing human and dog LV myocardium and assessed its interaction with Akt (cell-survival enzyme) and p38MAPK (programmed cell death enzyme).*

Methods: *Total RNA and Sodium-Dodecyl Sulfate (SDS) extracts were prepared from homogenate of LV specimens of 6 dogs with intracoronary microembolization-induced HF, 6 normal (NL) dogs, 7 explanted failed human hearts due to Idiopathic Dilated Cardiomyopathy (IDC), 7 failed human hearts due to Ischemic Cardiomyopathy (ICM) and 7 non-failing human donor hearts (DNR). SDS extracts were also prepared from cytosolic and mitochondrial fractions isolated from LV specimens.*

Results: *H11K mRNA and protein levels normalized to GAPDH increased significantly in LV tissue from ICM and IDC hearts compared to DNR hearts and in HF dogs compared with NL dogs. H11K protein levels increased in cytosolic fractions but decreased in mitochondrial fractions of both failed human and dog hearts compared to DNR hearts and NL dog hearts. Immunoprecipitation studies in specimens from HF dogs showed that H11K cytosolic fractions interacted predominantly with p38MAPK and least with Akt when compared with NL dogs.*

Conclusions: *Enhanced interaction of H11K with p38MAPK in HF can promote cell death thus contributing to progressive LV dysfunction. Therapeutic modalities that restore interaction of H11K with Akt and augment H11K translocation to mitochondria can potentially and partially reverse the progression of LV dysfunction by promoting cell survival.*

Keywords: *Heart Failure, H11Kinase, mRNA Expression, Mitochondria, Cytosolic Fraction*

Introduction

Despite a number of effective therapies, mortality and morbidity in patients with Heart Failure (HF) remains alarmingly high and reflects the need for more effective targeted therapies that address the underlying molecular defects that contribute to the progression of left ventricular dysfunction [1,2]. In addition to the well-recognized pathological signaling pathways such as calcineurin-NFAT3 [3,4] and downregulation of SERCA 2a activity [5] that can lead to worsening of LV function, the stress burden on the failing myocardium can lead to activation of several cell survival pathways that protect against cell loss [6,7]. One such pathway involves a serine/threonine kinase (H11 kinase [H11K]), a 22-kDa protein abundantly expressed in skeletal muscle and heart. Due to its homology with the C-terminal α-crystallin domain of the small heat-shock protein (Hsp) family, H11K is often referred to as HspB8 or Hsp22 [8]. H11K/HspB8/Hsp22 physically interacts with Hsp27 and, therefore, plays a cell-protective role in cardiac myocytes [8].

Although H11K has been implicated in inducing apoptosis of cardiomyocytes [9,10], its cardiac overexpression in mice showed enhanced cardiomyocyte survival [11] suggesting a functional duality. Cell survival by H11K overexpression is mediated through activation of the phosphoninositide 3-kinase (PI3K)-Akt-survival pathway [12]. In ischemia/reperfusion studies H11K was shown to represent a novel case of a dual-specificity kinase with reciprocal effects on cell death and survival depending on its subcellular localization, acting as a promoter of cell survival in the nucleus and mitochondria and as a tumor suppressor in the cytosol [10-14]. In the current study, H11K mRNA and protein levels in LV tissue, and subsequently in cytosolic and mitochondrial fractions isolated from LV specimens of normal (NL) and HF dogs and from explanted failed human hearts with Idiopathic Dialed Cardiomyopathy (IDC) or Ischemic Cardiomyopathy (ICM) as well as donor human hearts. The current study also examined the interaction of cytosolic H11K with Akt, a cell survival enzyme [12] and p38MAPK, cell programmed cell death enzyme [15].

Materials and methods

Procurement of LV tissue

LV tissue was obtained from 6 dogs with HF produced by multiple intracoronary microembolizations as described previously [16] and from 7 normal dogs. The study was approved by Henry Ford Health System Institutional Animal Care and Use Committee and conformed to the National Institute of Health "Guide and Care for Use of Laboratory Animals."Failed explanted human hearts were obtained from 7 patients with end-stage HF due to IDC and 7 patients with end-stage HF due to ICM. Immediately after excision, the failing hearts were placed in an ice-cold cardioplegic solution

and LV tissue (free of obvious scar or epicardial vessels) was cut in 5-mm3 blocks, quickly frozen in liquid nitrogen, and stored at -70°C until needed. LV specimens from 7 donor (DNR) human hearts deemed not suitable for transplantation were collected, prepared in a similar fashion as the failing hearts, and stored at -70°C until used. Studies in human tissue were approved by the Henry Ford Health System Institutional Review Board.

RNA isolation and real-time PCR

Total RNA was isolated from LV specimen and its integrity was determined electrophotometrically and spectrophotometrically as described previously [17,18]. A ratio of 1.7 at 260 nm/280 nm was considered good quality RNA and was used in the study. Primers, used in real-time Polymerase Chain Reaction (PCR) specific for H11K and the housekeeping gene glyceraldehyde-1, 3-diphosphate dehydrogenase (GAPDH) were designed using Primer Express Software (Version 2.0. Applied Biosystems) and synthesized by Operon Biotechnologies, Inc (Huntsville, AL). Reverse Transcription (RT) of isolated RNA was carried out with the high-capacity cDNA archive kit.Real-time PCR was performed with the SYBR Green PCR Master Mix (Super Array) using the ABI Prism 7550 Fast Sequence Detection System (Thermo Fisher Scientific, Waltham, MA). Product identity was confirmed by sequence analysis performed by the DNA sequencing facility at Wayne State University (Detroit, MI) and by electrophoresis on 1.6% agarose-ethidium bromide gel. For absolute quantification of H11K and GAPDH genes, standard curves were generated from varying amounts of the purified H11K and GAPDH cDNA. The threshold cycle (CT) was calculated and a plot between the CT values (Y-axis) and logarithm of H11K or GAPDH template concentration (X-axis) was constructed using SDS software (Thermo Fisher Scientific, Waltham, MA). The quality of the standard curve was judged from the slope and the correlation coefficient. This curve was used to calculate molecules mRNA of H11K and GAPDH and expressed as µg of total RNA.

Isolation of cytosolic and mitochondrial fractions

Mitochondria (MITO) from frozen LV tissue were isolated by differential centrifugation as described previously [19]. Briefly, LV homogenate was first centrifuged at 584 g for 10 min, and then the supernatant re-centrifuged at 9000 g for 10 min to collect mitochondrial pellet that was subsequently washed twice and finally suspended in KME buffer (100 mM KCl, 50 mM Mops, and 0.5 mM EGTA, pH 7.4). The supernatant from the above first spin at 9000 g was saved and then centrifuged at 100,000 g for 30 min. The resulting supernatant, referred to as cytosolic fraction, and pellets were aliquoted and stored at -70°C. MITO and cytosolic fraction protein concentration were determined by the Lowry method using bovine serum albumin as a standard.

Western Blotting of H11K

Homogenate, cytosolic and mitochondrial fractions from LV myocardium were prepared and their Sodium-Dodecyl Sulfate (SDS) extracts prepared as described previously [19]. Using Western blotting technique and specific antibodies, protein levels of H11K and GAPDH, an internal loading control were determined in homogenate and cytosolic fractions and H11K and porin, an internal loading control in mitochondrial fractions of SDS extract. [17,18]. Approximately 100 µg cytosolic fractions was immunoprecipitated with H11K primary antibody. The immunoprecipitate was fractionated on SDS-PAGE (sodium-dodecyl sulfate-polyacrylamide gel electrophoresis) and transferred on Millipore membrane filters, and then the blot was probed with phosphorylated (p-) and total (t-) p38MAPK and Akt, as well as H11K antibodies. Primary antibody of H11K (rabbit polyclonal, abcam, Cambridge, MA), GAPDH (mouse monoclonal, Fitzgerald, Acton, MA), porin (rabbit polyclonal, Sigma-Aldrich, St. Louis, MO), p38MAPK (goat polyclonal antibody, Santa Cruz Biotech, Dallas, TX), phosphorylated p38MAPK (mouse monoclonal antibody, Santa Cruz Biotech, Dallas, TX), Akt (rabbit polyclonal antibody, Cell Signaling Technology, Danvers, MA), and phosphorylated Akt (rabbit polyclonal antibody, New England Biolabs Ltd, Beverly, MA) were purchased. Dilution of primary antibody was carried out as outlined by the supplier. In all instances, the antibody was present in excess over the antigen, and the density of each protein band was in the linear scale. Band intensity was quantified using a gel densitometer (Bio-Rad Laboratories Inc., Philadelphia, PA) and expressed in densitometric units (du).

Statistical analysis

For studies in dog tissues, comparisons between HF and NL measures were made using a t-statistic for two means and significance set at $p < 0.05$.For studies in human tissues where 3 groups were present (DNR, ICM and ICD), measures were compared using one-way analysis of variance (ANOVA), with α set at 0.05. If significance was achieved, pairwise comparisons were performed using the Student-Neuman-Keuls test. For this test, a probability (P) value < 0.05 was considered significant. All data are expressed as means ± Standard Error of the Mean (SEM). The software package used for the above analysis was Primer of Biostatisticsby Glantz SA., Version 3.0, McGraw-Hill, Inc. 1992.

Results

H11K Sequencing and Quantitation of mRNA

Using specific forward and reverse primers, H11K gene was amplified and found to possess 201 bp as expected. Sequences of the amplified gene in LV tissue were identical between NL and HF dogs. Identical gene sequences were found in explanted human LV tissue obtained from DNR hearts and explanted failed human hearts due IDC or ICM. H11K mRNA molecules normalized to GAPDH were increased by nearly 4-fold in LV myocardium of HF dogs compared with NL dogs ($p < 0.05$; Figure 1). In human DNR and failing hearts due to ICM or IDC, the number of GAPDH mRNA molecules were similar. mRNA molecules of H11K normalized to GAPDH were significantly increased in human failing hearts regardless etiology compared with DNR hearts ($p < 0.05$ vs. DNR) (Figure 1).

H11K Protein Levels

Using polyclonal antibody, a prominent protein band of H11K with a molecular weight in vicinity of 22 kDa was observed on 12% SDS-polyacrymide gel. H11K protein level was significantly increased in LV homogenate of dogs with HF compared with NL dogs while the internal control GAPDH protein levels was unchanged (Figure 2). Similarly, H11K protein level was significantly higher in human failing hearts regardless etiology compared with DNR hearts (Figure 2).

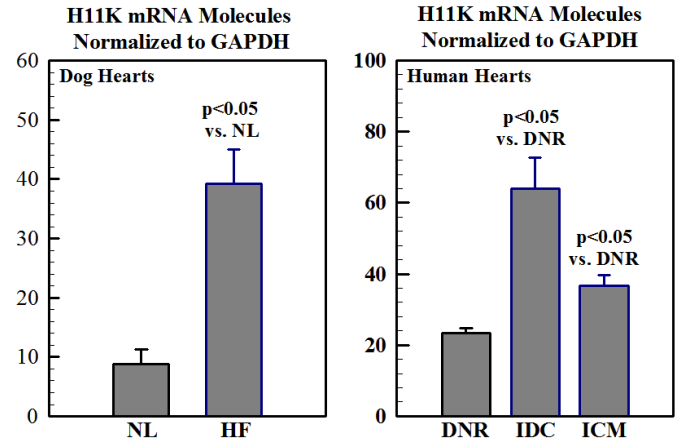

Figure 1. H11 kinase (H11K) mRNA molecules normalized to GAPDH

LEFT: Bar graphs depicting mean ± SEM of H11 kinase (H11K) messenger (m) ribonucleic acid (RNA) molecules/µg of total RNA normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA molecules/ µg of total RNA in left ventricular myocardium of normal (NL) dogs and dogs with heart failure (HF). RIGHT: Bar graphs depicting mean ± SEM of H11 kinase (H11K) mRNA molecules/µg of total RNA normalized to GAPDH mRNA molecules/µg of total RNA in Left Ventricular (LV) myocardium of human donor deemed not suitable for transplantation (DNR) hearts, explanted hearts from patients with Idiopathic Dilated Cardiomyopathy (IDC), and explanted hearts from patients with Ischemic Cardiomyopathy (ICM).

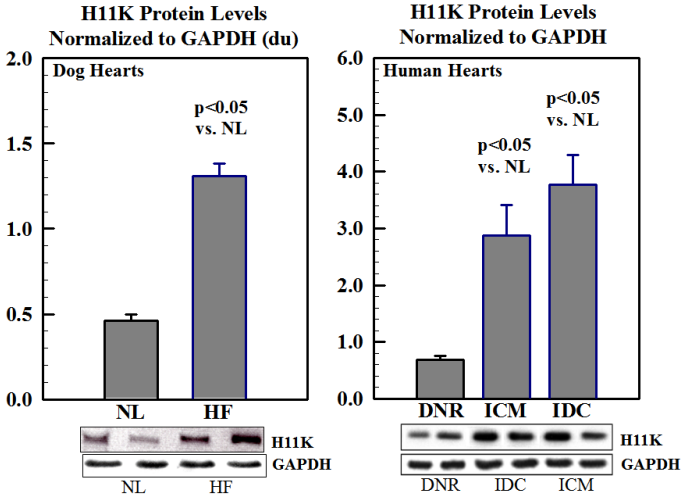

Figure 2. H11K and GAPDH proteins in LV homogenate

LEFT: Bar graphs depicting mean ± SEM of H11 kinase (H11K) protein levels normalized to Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) in left ventricular myocardium of normal (NL) dogs and dogs with Heart Failure (HF). Below the bar graph are Western blots showing H11K protein and GAPDH in 2 specimens of left ventricular homogenate from NL and HF dogs.

RIGHT: Bar graphs depicting mean ± SEM of H11 kinase (H11K) protein levels normalized to GAPDH in left ventricular myocardium of human donor (DNR) hearts, hearts from patients with Idiopathic Dilated Cardiomyopathy (IDC), and hearts from patients with Ischemic Cardiomyopathy (ICM). Below the bar graph are Western blots showing H11K protein and GAPDH in 2 specimens of left ventricular homogenate from DNR, IDC and ICM human hearts.

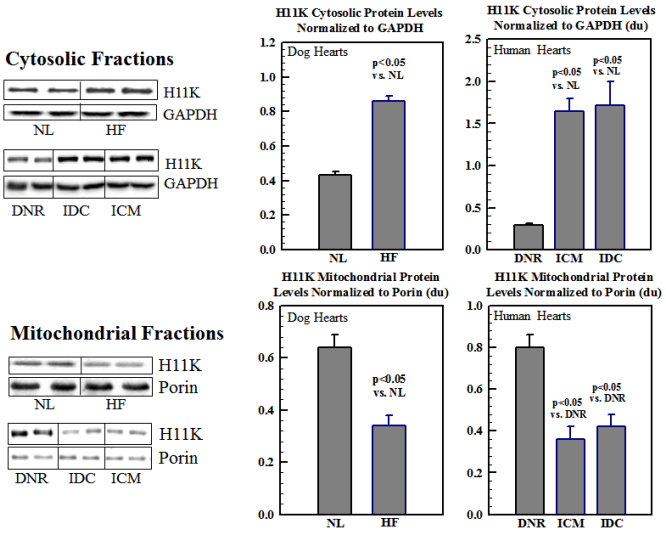

Figure 3. *H11 protein levels in cytosolic and mitochondrial fractions*

LEFT: Western blots showing H11K and Glyceraldehyde 3-Phosphate Dehydrogenase (GAPDH) in cytosolic fraction (top) and in mitochondrial fractions and porin (bottom). Bands are shown for 2 normal (NL) and 2 Heart Failure (HF) dogs and 2 bands each for human donor (DNR) hearts, hearts from patients with Idiopathic Dilated Cardiomyopathy (IDC), and hearts from patients with Ischemic Cardiomyopathy (ICM).

MIDDLE: Bar graphs depicting mean ± SEM of H11 kinase (H11K) protein levels in cytosolic fractions normalized to GAPDH (top) and in mitochondrial fractions normalized to porin (bottom) from NL dogs and dogs with HF.

RIGHT: Bar graphs depicting mean ± SEM of H11 kinase (H11K) protein levels in cytosolic fractions normalized to GAPDH (top) and in mitochondrial fractions normalized to porin (bottom) from DNR, IDC and ICM human hearts.

To further assess if an alteration in H11K protein levels occurs in the cytosolic and mitochondrial fractions, H11K protein levels were measured in both fractions, while GAPDH was used as internal control for the cytosolic fraction, and porin was used as a control for the mitochondrial fraction. H11K protein level was significantly reduced in mitochondrial fractions of dogs with HF compared to NL dogs (Figure 3). Similarly, H11K protein level was significantly reduced in human failing hearts regardless etiology compared with DNR hearts (Figure 3).In contrast, H11K protein level in cytosolic fractions was higher in dog and human failing hearts compared to NL and DNR hearts, respectively (Figure 3).

Interaction of H11K with Akt and p38MAPK

Cytosolic H11K interaction with t-p38MAPK or t-Akt in 3NL dogs and 3 HF dogs did not show any changes between NL and HF dogs (Figure 4). In contrast, H11K-immunoprecipitate of cytosolic fractions in the same 3 NL and 3 HF dogs exhibited high interaction of H11K with p-p38MAPK but lesser interaction with p-Akt in HF hearts compared to NL (Figure 4). The ratio of p-p38MAPK to t-p38MAPK was significantly higher in HF dogs compared with NL dog (Figure 4). In contrast, the ratio of p-Akt to t-Akt was significantly lower in HF dogs compared with NL dogs (Figure 4).

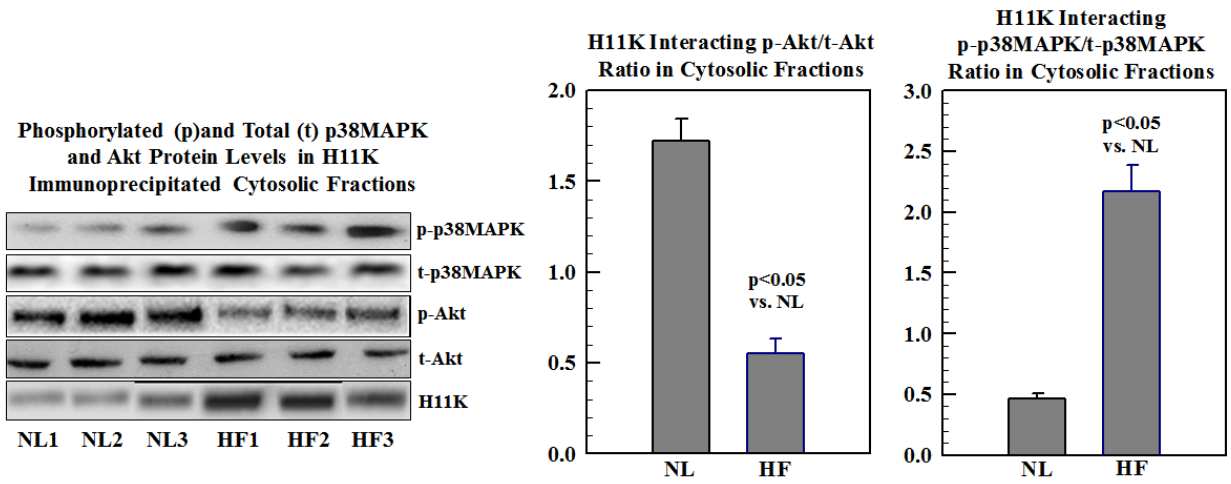

Figure 4. *Phosphorylated and total p38MAPK and Akt protein levels in H11K immunoprecipitated cytolsolic fractions*

LEFT: Western blots showing phosphorylated (p) and total (t) p38 mitogen activated protein kinase (p38MAPK) and protein kinase B (Akt) and H11 kinase (H11K) proteins in H11K-immunoprecipate from cytosolic fractions from left ventricular myocardium of 3 normal (NL1, NL2, NL3) and 3 heart failure (HF1, HF2, HF3) dogs.
MIDDLE: Bar graphs depicting mean ± SEM of ratio of p-Akt to t-Akt in cytosolic fractions of left ventricular myocardium from 3 NL dogs and 3 dogs with HF.
RIGHT: Bar graphs depicting mean ± SEM of ratio of p-p38MAPK to t-p38MAPK in cytosolic fractions of left ventricular myocardium from 3 NL dogs and 3 dogs with HF.

Discussion

In the current study, we tested the hypothesis that HF is a stress condition that is also associated with increased expression of H11K. H11K has been described as an "integrative sensor" in the cardiac adaptation to stress in that it coordinates cell growth, survival, and metabolism, the latter evidenced by the ability of H11K to increase glycogen synthesis [20]. In the present study, H11K levels were increased in LV of dogs and humans with HF compared to normal hearts. Furthermore, the distribution of H11K protein in the cytosolic and mitochondrial cellular fractions was altered in failing hearts compared to normal hearts. This endogenous up-regulation in LV cytosolic fractions is likely to promote program cell death through its interaction with p38MAPK [21,22]. In contrast, its endogenous down-regulation in mitochondrial fractions is likely to promote mitochondrial dysfunction through opening of the mitochondrial permeability transition pore [23,24].

Most studies exploring the role of H11K in cell growth and survival have been performed in ischemia-reperfusion studies conducted in transgenic mice that overexpress the protein in the heart or delete the protein with knock-out approaches [10-12]. In one such study in which H11K was overexpressed in the heart by 7-fold using a cardiac specific α-MHC promoter, the fetal gene program was re-expressed and LV concentric hypertrophy with preserved contractile function was observed [9]. Studies in cardiac myocytes in which an adenovirus harboring H11-KI (kinase-inactive mutant of H11) and LY294002, an inhibitor of PI3K were used, the study concluded that myocytes hypertrophy was induced by H11K-independent Akt-pathway leading to cell survival whereas H11K-dependent p38MAPK activation led to cardiomyocyte apoptosis [21]. Previous studies have also suggested that H11K protein is required for proper function of the heart and its deletion in the mouse heart caused less cardiac hypertrophy along with LV dilation, impaired contractile function, increased interstitial collagen, faster transition into HF, and increased mortality in response to pressure overload [12]. Studies such as these clearly suggest that H11K protein in the normal heart is cardioprotective [12]. Other studies have shown that overexpression of H11K in the cytosolic compartment of LV myocardium inhibits Akt and p70S6K and promotes cell death whereas overexpression in the nucleus exerts cytoprotective effect [10]. A dual role of H11K was also suggested namely acting as a chaperone promoting cardiac cell growth and survival through the interaction with TAK1-PI3K-Akt pathway, and a kinase promoting apoptosis through phosphorylation of p38-mitogen activated protein kinase (p38-MAPK) [22]. A recent study in a H11K knockout mouse suggested that H11K is required for proper cardiac mitochondrial oxidative phosphorylation through regulation of the transcription factor signal transducer and activator of transcription 3 (STAT3) [12]. Thus, the existing knowledge to date clearly suggests that the pro-survival versus pro-death effects of H11K crucially depend on the balance between p38MAPK, Akt activity, and STAT3 transcription. What remains unclear is the role of H11K in the failing heart. Our observations in failing hearts indicate that cytosolic H11K interacts predominantly with p38MAPK and least with Akt. These findings suggest that the cell death pathway is activated in the failing heart while pro-survival pathways are inhibited. Furthermore, reduced

H11K levels in mitochondria isolated from failing hearts may trigger mitochondrial dysfunction through reduced oxidative phosphorylation, a dysfunction observed in HF [19]. Reduced H11K in mitochondria suggests that post translation, such as acetylation, sumoylation and proteolysis of mitochondrial binding domain of H11K at its n-terminal may allow H11K translocation from mitochondria to cytosol. Promoting exogenous-mediated over-expression of H11K specifically in mitochondria of failing hearts may offer a novel opportunity for treating chronic HF. Improving H11K expression in mitochondria of the failing heart is likely to enhance oxidative phosphorylation [19] and improve cardiomyocyte survival by inhibiting pro-apoptotic pathways.

Conclusions

The failing LV manifests increased expression of H11K in both human and dog hearts. In humans the increased expression is independent of heart failure etiology. Furthermore, the reduced H11K protein in mitochondria and its increase in the cytosolic compartment of failing cardiomyocytes represents an adverse maladaptation capable of reducing ATP synthesis by mitochondria and activation of pro-apoptotic pathways in the cytosol respectively. These finding support a role for maladaptations of H11K in the progression of HF. Further studies are needed to understand the nature of translocation of H11K from cytosol to mitochondria in failing hearts and explore approaches that reverses this translocation. Studies such as these will allow for the development of a therapy for selective increase of H11K levels in mitochondria of failing heart.

References

1. Abraham WT, Gilbert EM, Lowes BD, Minobe WA, Larrabee P, et al. Coordinate changes in Myosin heavy chain isoform gene expression are selectively associated with alterations in dilated cardiomyopathy phenotype. *Mol Med.* 2002; 8: 750-760.
2. Lowes BD, Gilbert EM, Abraham WT, Minobe WA, Larrabee P, et al. Myocardial gene expression in dilated cardiomyopathy treated with beta-blocking agents. *N Engl J Med.* 2002; 346: 1357-1365.
3. Fiedler B, Lohmann SM, Smolenski A, Linnemuller S, Pieske B, et al. Inhibition of calcineurin-NFAT hypertrophy signaling by cGMP-dependent protein kinase type I in cardiac myocytes. *Proc Natl Acad Sci USA.* 2002; 99: 11363-11368.
4. Nakayama H, Wilkin BJ, Bodi I, Molkentin JD. Calcineurin-dependent cardiomyopathy is activated by TRPC in the adult mouse heart. *FASEB J.* 2006; 20: 1660-1670.
5. Gupta RC, Mishra S, Rastogi S, Sharov VG, Sabbah HN. Improvement of cardiac sarcoplasmic reticulum calcium cycling in dogs with heart failure following long-term therapy with the Acorn Cardiac Support Device. *Heart Fail Rev.* 2005; 10: 149-155.
6. Jeong D, Cha H, Kim E, Kang M, Yang DK, et al. PICOT inhibits cardiac hypertrophy and enhances ventricular function and cardiomyocyte contractility. *Circ Res*. 2006; 99: 307-314.
7. Palfi A, Toth A, Hanto K, Deres P, Szabados E, et al. PARP inhibition prevents postinfarction myocardial remodeling and heart failure via the protein kinase C/glycogen synthase kinase-3beta pathway. *J Mol Cell Cardiol*. 2006; 41: 149-159.
8. Christians ES, Yan LJ, Benjamin IJ. Heat shock factor 1 and heat shock proteins: Critical partners in protection against acute cell injury. *Crit Care Med.* 2002; 30: S43-50.
9. Depre C, Hase M, Gaussin V, Zajac A, Wang L, et al. H11 kinase is a novel mediator of myocardial hypertrophy in vivo. *Circ Res*. 2002; 91: 1007-1014.
10. Julie A, Maria K, Rocchi P. Small heat shock proteins HSP27 (HspB1), αB-crystallin (HspB5) and HSP22 (HspB8) as regulators of cell death. *The International Journal of Biochemistry & Cell Biuology.* 2012, 44: 1622-1631.
11. Depre C, Wang L, Sui X, Qiu H, Hong C, et al. H11 kinase prevents myocardial infarction by preemptive preconditioning of the heart. *Circ Res.* 2006; 98: 280-288.
12. Qiu H, Lizano P, Laure L, Sui X, Rashed E, et al. H11 kinase/heat shock protein 22 deletion impairs both nuclear and mitochondrial functions of STAT3 and accelerates the transition into heart failure on cardiac overload. *Circulation.* 2011; 124: 406-415.
13. Depre C, Kim SJ, John AS, Huang Y, Rimoldi OE, et al. Program of cell survival underlying human and experimental hibernating myocardium. *Circ Res.* 2004; 95: 433-440.
14. Marunouchi T, Abe Y, Murata M, Inomata S, Sanbe A, et al. Changes in small heat shock proteins HSPB1, HSPB5 and HSPB8 in mitochondria of the failing heart following myocardial infarction in rats. *Biol. Pharm. Bull.* 2013; 36: 529-539.
15. Heusch P, Canton M, Aker S, van de Sand A, Konietzka I, et al. The contribution of reactive oxygen species and p38 mitogen-activated protein kinase to myofilament oxidation and progression of heart failure in rabbits. *Br J Pharmacol.* 2010; 160: 1408-1416.
16. Sabbah HN, Stein PD, Kono T, Gheorghiade M, Levine TB, et al. A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. *Am J Physiol.* 1991; 260: H1379-384.
17. Gupta RC, Mishra S, Rastogi S, et al. Cardiac SR-coupled PP1 activity and expression are increased and inhibitor-1 protein expression is decreased in failing hearts. *Am J Physiol Heart Sirc Physiol.* 2003; 285: H2373-2381.
18. Gupta RC, Mishra S, Yang XP, Sabbah HN. Reduced inhibitor 1 and 2 activity is associated with increased protein phosphatase type 1 activity in left ventricular myocardium of one-kidney, one-clip hypertensive rats. *Mol Cell Biochem*. 2005; 269: 49-57.
19. Sabbah HN, Gupta RC, Kohli S, Wang M, Hachem S, et al. Chronic therapy with elamipretide (MTP-131), a novel mitochondria-targeting peptide, improves left ventricular and mitochondrial function in dogs with advanced heart failure. *Circ Heart Fail.* 2016; 9: 1-24.
20. Wang L, Zajac A, Hedhli N, Depre C. Increased expression of H11 kinase stimulates glycogen synthesis in the heart. *Mol Cell Biochem*. 2004; 265: 71-78.
21. Hase M, Depre C, Vatner SF, Sadoshima J. H11 has dose-dependent and dual hypertrophic and proapoptotic functions in cardiac myocytes. *Biochem J.* 2005; 388: 475-483.
22. Sui X, Li D, Qiu H, Gaussin V, Depre C. Activation of the bone morphogenetic protein receptor by H11kinase/Hsp22 promotes cardiac cell growth and survival. *Circ Res.* 2009; 104: 887-895.
23. Laure L, Long R, Lizano P, Zini R, Berdeaux A, et al. H11 kinase/Hsp22 stimulates oxidative phosphorylation and modulates mitochondrial reactive oxygen species production: Involvement of a nitric oxide-dependent mechanism. *Free Radic Biol Med*. 2012, 52: 2168-2176.
24. Chen YR, Zweier JL. Cardiac mitochondria and reactive oxygen species generation. *Circ Res.* 2014, 114: 524-537.

Correspondence: Ramesh C. Gupta, PhD, Cardiovascular Research, Henry Ford Health System, Department of Medicine, Division of Cardiovascular Medicine, Henry Ford Hospital, 2799 West Grand Boulevard, Detroit, Michigan 48202, USA, Tel: (313) 916-7363, Fax: (313) 916-3001, E-mail: rgupta1@hfhs.org

Rec: Aug 10, 2019; Acc: Sep 08, 2019; Pub: Sep 12, 2019

J Cardio Res. 2019;2(2):21
DOI: gsl.jcr.2019.000021