HSP90 inhibitor 17-AAG prevents apoptosis of cardiomyocytes via miR-93–dependent mitigation of endoplasmic reticulum stress

Jingjing Guo | Shengnan Li | Yanming Li | Chenyun Yan | Qilin Wan | Zhizhong Wang

Department of Cardiology, Huaihe Hospital, Henan University, Kaifeng City, Henan, China

Correspondence
Yanming Li, Department of Cardiology, Huaihe Hospital, Henan University, No. 8 Baobei Road, Kaifeng City, 475000 Henan Province, China. Email: nmjoej9@163.com

Abstract
Heart failure accounts for substantial morbidity and mortality worldwide. Accumulating evidence suggests that aberrant cardiac cell death caused by endoplasmic reticulum stress (ERS) is often associated with structural or functional cardiac abnormalities that lead to insufficient cardiac output. The detailed molecular mechanism underlying the pathological death of cardiomyocytes still remains poorly understood. We found that 17-AAG (tanespimycin), an HSP90 (heat shock protein 90) inhibitor often used to kill cancer cells, could potently inhibit tunicamycin-induced ERS and the downstream nuclear factor kappa B activity in neonatal rat cardiomyocytes, leading to diminished apoptotic signaling and thus enhanced cell survival. Interestingly, the antiapoptotic effect of 17-AAG on cardiomyocytes required normal expression of miR-93, an oncogenic microRNA known to promote cell survival and growth. Our study implicated a new pharmacological role of 17-AAG in supporting the miR-93–associated oncogenic signaling to prevent the pathological death of cardiomyocytes. The results opened opportunities for exploring new strategies in the development of therapeutic agents.

Keywords
17-AAG, cardiomyocytes, HSP90, miR-93

1 | INTRODUCTION
Heart failure is a pathological condition manifested by elevated intracardiac pressures or an insufficient cardiac output. It is a rapidly growing public health issue with an estimated prevalence of >37.7 million individuals globally, largely because of aging of the population.1,2 There are a variety of known risk factors linked to the disease including but not limited to age, diet, sex, and genetic predisposition. At the molecular level, programmed cell death of cardiac muscle cells has been identified as an essential process in the progression to heart failure.3,4 Various antiapoptotic agents thus have become the focus of research works aimed to the development of new intervening approaches.5

Accumulating evidence demonstrates that endoplasmic reticulum stress (ERS) is a key contributor to apoptotic cell losses in the pathogenesis of many types of cardiovascular diseases including heart failure.6,7 Tunicamycin (TM) is a mixture of homologous nucleoside antibiotics that can block N-linked glycosylation in treated cells. It has been used to induce ERS in cultured neonatal rat cardiomyocytes, helping create in vitro apoptosis models.8,9 These have facilitated the
identification of reagents that can inhibit ERS and prevent aberrant apoptotic loss of cardiomyocytes.\textsuperscript{10-12} Interestingly, an HSP90 (heat shock protein 90) inhibitor, 17-AAG (tanespimycin), has emerged as a new antiapoptotic agent in different types of cells.\textsuperscript{13-15} This inhibitor regulates a highly complex molecular network and the outcome of the treatment can be influenced by different cellular and physiological contexts. The exact effects of 17-AAG on ERS-linked apoptosis of cardiomyocytes still remain ambiguous.

The complexity of the cell signaling in controlling apoptosis can be further demonstrated by the action of nuclear factor kappa B (NF-\( \kappa \)B), a transcriptional complex that can be activated by ERS in cardiac cells.\textsuperscript{16-18} When unstimulated, NF-\( \kappa \)B, such as a predominant p65/p50 heterodimer, is held in the cytoplasm by a family of IkB inhibitors. Activation of the complex is initiated by phosphorylation and degradation of the inhibitors, such as IkB\( \alpha \), the primary inhibitor of p65/p50.\textsuperscript{19} NF-\( \kappa \)B is then freed to enter the nucleus where it can trigger the expression of specific genes regulating processes including cell survival, apoptosis, inflammation, and antiviral responses.\textsuperscript{19-21} Although prolonged activation of NF-\( \kappa \)B can contribute to pathogenesis by promoting cardiac cell death, it appears to protect cardiovascular tissues from injuries under many conditions.\textsuperscript{16,22}

Here, we report a new pharmacological role of 17-AAG in cardiomyocytes. We found that 17-AAG could inhibit TM-induced ERS and NF-\( \kappa \)B activation in neonatal rat cardiomyocytes, and ultimately prevent the cells from apoptosis. The protective effect of 17-AAG required normal expression of miR-93, which is potentially an important regulator of cardiac cell fate under stress conditions.\textsuperscript{23,24} Further characterization of the functional link between 17-AAG and miR-93 will likely help develop new therapeutic agents to prevent the progression of heart failure.

2 | MATERIALS AND METHODS

2.1 | Animals and antibodies

One- to three-day-old Sprague-Dawley (SD) rats, weighted between 180 and 200 g, were used in this study. All animal procedures were complied with the Animal Management Rule of the Ministry of Health, People’s Republic of China (documentation no. 55, 2001) and the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and approved by the Animal Care Committee of Henan University.

The primary antibodies targeting x, y, and z were obtained from Kirkegaard & Perry Laboratory. Each test in this study was carried out triplically.

2.2 | Isolation and culture of neonatal rat cardiomyocytes

All steps were performed in a sterile cell-culture hood by following a previously described protocol with minor modifications.\textsuperscript{24} Hearts were extracted from the 1- to 3-day-old SD rats and transferred immediately into a dish on ice. The hearts were washed and minced into small pieces in Dulbecco modified Eagle medium (DMEM, Hyclone) containing 25 mM \( \Delta \)glucose and 4 mM \( \Delta \)glutamate. The tissue fragments were transferred into a tube and trypsin was added for 10 minutes digestion at 37°C. The supernatant was transferred to a new tube and 10% fetal bovine serum (FBS) was supplemented to stop the digestion. The remaining tissue was digested again for 10 minutes at 37°C. This was repeated until all tissue was digested. The cells were gently aspirated and pooled in a new tube. After being harvested by centrifugation, the cells were resuspended and plated at a density of 10\(^5\) cells/cm\(^2\). Cardiomyocytes were enriched by incubating the cells in DMEM containing 0.1 mM bromodeoxyuridine (Sigma-Aldrich) at 37°C for 72 hours. The cardiomyocytes were maintained in DMEM supplemented with 10% FBS (Hyclone) at 37°C in humidified 5% CO\(_2\) unless specified elsewhere.

2.3 | Induction and prevention of apoptosis

TM and 17-AAG (tanespimycin) were obtained from Cayman Chemical. The neonatal cardiomycocytes were starved in the serum-free DMEM for 24 hours before being treated with specified compounds. To induce apoptosis, the cultured neonatal cardiomyocytes were cultured in the presence of 1 nM TM for 24 hours. To prevent apoptosis, 1 \( \mu \)M 17-AAG was added to the cultured cells 30 minutes before TM treatment.

2.4 | Cell viability assay

MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) assays were performed to determine cell viabilities. Briefly, neonatal rat cardiomyocytes were seeded in a 96-well culture plate at the density of 2 \( \times \) 10\(^4\) cells/well. The cells were exposed to TM in the presence or absence of 17-AGG for 24 hours. Cell viabilities were determined by measuring the cellular NAD(P)H-dependent oxidoreductase activities against a yellow tetrazolium salt, MTT (Sigma-Aldrich). The amounts of deep purple
products were determined by measuring absorbance at 490 nm.

2.5 | TUNEL assay

DNA fragmentation was detected in situ by using a TUNEL fluorescence kit (Roche). Briefly, cardiomyocytes grown on coverslips were washed with PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na2HPO4, 1.4 mM KH2PO4, pH 7.4), and fixed in a 4% paraformaldehyde solution for 1 hour at 4°C. The cells were permeabilized by 0.1% Triton X-100 for 2 minutes, and incubated in a freshly prepared TUNEL reaction mixture for 1 hour at 37°C in the dark. The coverslips were then washed with PBS, and mounted on slides with an antifading solution. The TUNEL staining results were analyzed with an Eclipse 80i fluorescence microscope (Nikon).

2.6 | Western blot analysis

Proteins were detected and quantitated by Western blot analysis according to previously described methods.23 Cultured primary cardiomyocytes were washed with PBS, harvested by scraping and centrifugation, and then resuspended in a radioimmunoprecipitation assay buffer (10⁶ cells/µL). Equal amounts of proteins were loaded onto 10% sodium dodecyl sulfate gels. After electrophoretic separation, the in-gel proteins were transferred onto nitrocellulose membranes. The membranes were then blocked by 5% nonfat dried milk (1 hour at room temperature) before they were sequentially incubated with specific primary antibodies (overnight at 4°C) and horse-radish peroxidase-conjugated secondary antibodies (1 hour at room temperature). The antibody detected protein bands were visualized by enhanced chemiluminescence (Multi-Sciences Biotech, China). β-Actin or histone H3 was blotted as an internal reference. Protein expression levels were assessed by the NIH the ImageJ software. All results were repeated at least three times.

2.7 | Quantitative real-time polymerase chain reaction

Total RNA samples were extracted using Trizol (Invitrogen) from cultured cardiomyocytes. Quantitative real-time polymerase chain reaction (RT-qPCR) was performed using mirVana microRNA (miRNA) Detection Kit (Ambion) to quantify miR-93 levels with the following primers. The levels of miR-93 were normalized to U6 snRNA using the 2−ΔΔCt method.

5′-AAGTGCTGTTCGTGCAGGT-3′ (forward);
5′-CTCGGGAAGTGTAGCTCA-3′ (reverse).

2.8 | Transfection

The anti-miR-93 inhibitor (5′-CUACCUGCACGAA-CAGCACUUUG-3′) and scrambled RNA control were synthesized by Ribo Bio in China. Cells were grown in multiwell plates. The RNAs were transfected into the cells at a final concentration of 100 nmol/L using Lipofectamine 2000 according to the manufacturer’s instructions (Invitrogen). The cells were harvested at 48 hours after transfection for the downstream assays.

2.9 | Statistical analysis

Statistical analysis was done with the GraphPad software. Data were presented as mean ± standard deviation (SD). Unpaired Student t test was used to compare the means between two groups. One-way analysis of variance followed by Newman-Keuls test was used to compare the means among three or more groups. Statistical significance was determined by P < 0.05.

3 | RESULTS

3.1 | Pretreatment of 17-AAG reduced TM-induced ER stress and nuclear NF-κB p65

Accumulating evidence demonstrates that ERS-induced apoptosis is the key contributor to cell loss in the pathogenesis of a series of cardiovascular diseases.6 To model ERS in the neonatal rat cardiomyocytes, the cells were treated with the TM compound and several proapoptotic proteins, GRP78, CHOP, caspase-12, and the active form of caspase-12, were indeed upregulated. However, when the cells were pretreated with 17-AAG, an HSP90 inhibitor, the expression levels of those proteins remained at the near normal levels (Figure 1A).

ERS has been found to activate NF-κB, which is normally held inactive by its inhibitor IκB. In the cardiomyocytes with TM-induced ERS, IκB was hyperphosphorylated and presumably subjected to proteasome-mediated degradation. As a result, NF-κB p65 was translocated into the nucleus, where the transcriptional factor could reshape the gene expression profile of the cardiomyocytes (Figure 1B,C). In contrast, when the cells were pretreated with 17-AAG, there was no apparent loss of IκB or enhanced accumulation of nuclear NF-κB p65 (Figure 1B,C).

3.2 | 17-AAG inhibited apoptosis of cardiomyocytes

We then try to find out if the pretreatment of 17-AAG could ultimately protect the neonatal cardiomyocytes
from apoptosis. We found that TM treatment led to enhanced apoptosis as suggested by reduced cell viability, elevated caspase-3 activity, and enhanced cellular DNA fragmentation. In contrast, when the cells were pre-treated with 17-AAG, the TM-induced apoptotic effects seem to be reversed. The cell viability, caspase-3 activity, and DNA fragmentation remained at the near normal levels. The results strongly supported that 17-AAG can efficiently protect cardiomyocytes from pathological apoptosis in many heart diseases. (Figure 2)

FIGURE 1 Tanespimycin (17-AAG) reduced tunicamycin (TM)-induced endoplasmic reticulum stress (ERS) and NF-κB activation in neonatal rat cardiomyocytes. A, Representative Western blot analysis of ERS related proteins (left) and statistical comparison of averaged protein expression levels (right). B, Representative Western blot analysis of proteins in the NF-κB pathway (left) and the statistical comparison of averaged protein levels (right). C, Immunofluorescence staining of NF-κB p65 in the cytoplasm and the nucleus. Scale bars: 100 μm. Three groups of cells are untreated (control), TM-treated (TM), and TM and 17-AAG treated (TM + 17-AAG), respectively. The levels of the control groups were normalized to 1. N = 6, mean ± SD, "P < 0.01 vs the control group; **P < 0.01 vs TM. NF-κB, nuclear factor kappa B

3.3 | Expression of miR-93 is affected by TM and 17-AAG

Recent studies have found that miR-93 can regulate cardiomyocyte apoptosis induced under different stress conditions.
conditions, such as those caused by ischemia/reperfusion and oxygen-glucose deprivation/reoxygenation injuries.23,24 Our preliminary study comparing global miRNA expression profiles found differential expression of a series of miRNAs including miR-93. We thus compared the expressions of miR-93 in neonatal rat cardiomyocytes under different treatment conditions. Interestingly, its expression level was significantly reduced in TM-treated cells, but not affected if the cells were pretreated with 17-AAG to prevent apoptosis (Figure 3). The result suggested that miR-93 may be important in protecting cardiomyocytes under various stress conditions including TM-induced ERS and its regulatory function is potentially linked to that of 17-AAG.

3.4 | miR-93 is required for 17-AAG to suppress ERS and NF-κB p65

To further confirm the importance of miR-93 in 17-AAG-mediated protection of cardiomyocytes, we tested whether downregulation of miR-93 could impair the functional impact of 17-AAG. As expected, transfection of anti-miR-93, an antisense RNA molecule, efficiently blocked miR-93 in the neonatal rat cardiomyocytes (Figure 4). While 17-AAG could prevent the TM-treated cells from upregulating the ERS-linked proapoptotic factors, GRP78, CHOP, caspase-12 and active-caspase-12, the protective impact disappeared in those cells with reduced miR-93 (Figure 5A). In addition, treating those cells with 17-AAG could not promote phosphorylation and degradation of I-κBα, leading to
sustained inhibition of the translocation of NF-κB p65 into the nucleus (Figure 5B,C).

3.5 | miR-93 is required for 17-AAG to suppress TM-induced apoptosis

We next examined whether the loss of miR-93 in cardiomyocytes could affect apoptosis when the cells were under the condition of TM-induced ERS. As expected, the cells with downregulation of miR-93 did not respond to the pretreatment of 17-AAG, as those cells showed similar apoptotic phenotypes as the cardiomyocytes treated by TM only, such as the reduced viability, elevated caspase-3 activity and enhanced DNA fragmentation level (Figure 6). Overall, the results strongly supported that 17-AAG can protect cardiomyocytes under the TM-mediated stress condition and its action is likely dependent on miR-93-associated regulatory networks.

4 | DISCUSSION

Our study strongly supported that 17-AAG can suppress TM-induced ERS in neonatal rat cardiomyocytes and ultimately downregulate apoptotic signaling to promote cell survival. The inhibitor was originally developed to inhibit HSP90 for the treatment of cancer, especially leukemia, and kidney tumors. It demonstrated significant and durable responses with low toxicity in phase II studies. Although 17-AAG can promote apoptosis of a variety of tumor cells, its pharmacological function in nontumor cells remains ambiguous. In fact, previous studies showed that treating 17-AAG on cultured cardiac cells led to different cell fates. Our results confirmed its antiapoptotic function in rat neonatal cardiomyocytes. Probably, the TM-induced apoptotic signaling is predominant in the stressed cells and thus the proapoptotic effect of 17-AAG is diminished, although other possibilities, such as inefficient inhibition of HSP90 in cardiomyocytes, cannot be ruled out.

The molecular basis of 17-AAG-mediated protection of cardiomyocytes under apoptotic stress remains largely unexplored. Our study suggested that miR-93 is an important downstream regulator for 17-AAG to reverse the apoptosis processes. In fact, miR-93 is best known as an oncogenic microRNA. Its expression is upregulated in a variety of tumors to presumably promote cancer cell survival and proliferation. Our preliminary analysis of miRNA libraries derived from cardiomyocytes under different conditions found differential expression of a series of candidates including miR-93. Notably, recent studies have also implicated its critical role in regulating apoptosis of cardiomyocytes that suffered different types of injuries, although its regulatory action seemed to render different outcomes probably because of the divergence of the associated signaling network. Our research thus demonstrated a new pharmacological role of 17-AAG in supporting oncogenic signaling in cardiac cells, which ultimately can protect the cells from pathological stress.

Patients with heart failure have been substantially benefited from the major advances in our understanding of the pathophysiology of the syndrome and development of a variety of treatment paradigms, which can often relieve symptoms and stop or slow the gradual
worsening of the condition. However, there are still major unmet medical needs and the prevalence of heart failure is expected to increase as population ages. Efforts to establish novel treatment approaches have been largely impeded by unwanted clinical outcomes. Optimizing clinical trial strategies and improving understanding of the causal mechanisms of the diseases are thus required to stimulate efforts in drug development. In this regard, our research may open new opportunities for further exploration of the critical biological processes responsible for the progression of heart failure caused by aberrant cell death, and thus will ultimately help formulate new promising therapeutic strategies.

**FIGURE 5** Inhibition of miR-93 diminished the reversing impacts of 17-AAG on TM-induced ERS and NF-κB activation. A, Representative Western blot analysis of ERS related proteins (left) and the statistical comparison of protein expression levels (right). B, Representative Western blot analysis of proteins in the NF-κB pathway (left) and the statistical comparison of averaged protein levels (right). C, Immunofluorescence staining of NF-κB p65 in the cytoplasm and the nucleus. The scale bars are 100 μm. The TM, TM + 17-AGG, and TM + 17-AGG + anti-miR-93 are cells treated with the annotated compounds and miRNA inhibitors. The anti-miR-93 inhibitors were transfected into the cells. After 24 hours, the cells were further treated with TM and 17-AAG. The levels of the control groups were normalized to 1. N = 6, mean ± SD, **P < 0.01 vs control; ***P < 0.01 vs TM. ERS, endoplasmic reticulum stress; miRNA, microRNA; NF-κB, nuclear factor kappa B; TM, tunicamycin.
FIGURE 6  The antiapoptotic effect of 17-AAG was diminished by anti-miR-93 treatment. A, Bar plot comparing the viability of cardiomyocytes. Cultured neonatal rat cardiomyocytes were exposed to compounds for 24 hours and then the cell viability was determined by MTT assays. B, Bar plot comparing caspase-3 activities in cardiomyocytes. C, Bar plot comparing DNA fragmentation levels in cardiomyocytes. D, Representative images of TUNEL-stained cells. The scale bars are 100 μm. The control represents untreated cells. The TM, TM + 17-AGG, and TM + 17-AGG + anti-miR-93 are cells treated with the annotated compounds and miRNA inhibitors. The anti-miR-93 inhibitors were transfected into the cells. After 24 hours, the cells were further treated with TM and 17-AAG. The levels of the control groups were normalized to 1. N = 6, mean ± SD, "P < 0.01 vs control; "#P < 0.01 vs TM. miRNA, microRNA

ORCID

Yanming Li http://orcid.org/0000-0002-0187-010X

REFERENCES

1. Ziaeian B, Fonarow GC. Epidemiology and aetiology of heart failure. Nat Rev Cardiol. 2016;13(6):368-378.
2. Metra M, Teerlink JR. Heart failure. Lancet. 2017;390(10106):1981-1995.
3. Chiong M, Wang ZV, Pedrozo Z, et al. Cardiomyocyte death: mechanisms and translational implications. Cell Death Dis. 2011;2:e244.
4. Vanempele V, Bertrand A, Hostra L, Crijns H, Doevendans P, Dewindt L. Myocyte apoptosis in heart failure. Cardiovase Res. 2005;67(1):21-29.
5. Singh SS, Kang PM. Mechanisms and inhibitors of apoptosis in cardiovascular diseases. Curr Pharm Des. 2011;17(18):1783-1793.
6. Minamino T, Kitakaze M. ER stress in cardiovascular disease. J Mol Cell Cardiol. 2010;48(6):1105-1110.
7. Sano R, Reed JC. ER stress-induced cell death mechanisms. Biochim Biophys Acta. 2013;1833(12):3460-3470.
8. Okada K, Minamino T, Tsukamoto Y, et al. Prolonged endoplasmic reticulum stress in hypertrophic and failing heart after aortic constriction: possible contribution of endoplasmic reticulum stress to cardiac myocyte apoptosis. Circulation. 2004;110(6):705-712.
9. Shen M, Wang L, Guo X, et al. A novel endoplasmic reticulum stress-induced apoptosis model using tunicamycin in primary cultured neonatal rat cardiomyocytes. Mol Med Rep. 2015;12(4):5149-5154.
10. Shen M, Wang L, Yang G, et al. Baicalin protects the cardiomyocytes from ER stress-induced apoptosis: inhibition
of CHOP through induction of endothelial nitric oxide synthase. *PLoS One.* 2014;9(2):e88389.

11. Lee JH, Kwon EJ, Kim DH. Calumenin has a role in the alleviation of ER stress in neonatal rat cardiomyocytes. *Biochem Biophys Res Commun.* 2013;439(3):327-332.

12. Wang Y, Xu L, Cui X, et al. Ibutilide treatment protects against ER stress induced apoptosis by regulating calumenin expression in tunicamycin treated cardiomyocytes. *PLoS One.* 2017;12(4):e0173469.

13. Naito AT, Okada S, Minamino T, et al. Promotion of CHIP-mediated p53 degradation protects the heart from ischemic injury. *Circ Res.* 2010;106(11):1692-1702.

14. Wagatsuma A, Takayama Y, Hoshino T, et al. Pharmacological targeting of HSP90 with 17-AAG induces apoptosis of myogenic cells through activation of the intrinsic pathway. *Mol Cell Biochem.* 2018;445:45-58.

15. Wang M, Sun G, Du Y, et al. Myricitrin protects cardiomyocytes from hypoxia/reoxygenation injury: involvement of heat shock protein 90. *Front Pharmacol.* 2017;8:353.

16. Van der heiden K, Cuhlmann S, Luong LA, Zakkar M, Evans PC. Role of nuclear factor kappaB in cardiovascular health and disease. *Clin Res.* 2010;118(10):593-605.

17. Dhingra R, Shaw JA, Aviv Y, Kirshenbaum LA. Dichotomous actions of NF-kappaB signaling pathways in heart. *J Cardiovasc Transl Res.* 2010;3(4):344-354.

18. Rivera-Serrano EE, Sherry B. NF-kappaB activation is cell type-specific in the heart. *Virology.* 2017;502:133-143.

19. Chen LF, Greene WC. Shaping the nuclear action of NF-kappaB. *Nat Rev Mol Cell Biol.* 2004;5(5):392-401.

20. Rubio D, Xu RH, Remakus S, et al. Crosstalk between the type 1 interferon and nuclear factor kappa B pathways confers resistance to a lethal virus infection. *Cell Host Microbe.* 2013;13(6):701-710.

21. Hayden MS, Ghosh S. NF-kappa B, the first quarter-century: remarkable progress and outstanding questions. *Genes Dev.* 2012;26(3):203-234.

22. Gordon JW, Shaw JA, Kirshenbaum LA. Multiple facets of NF-kappaB in the heart: to be or not to NF-kappaB. *Circ Res.* 2011;108(9):1122-1132.

23. Yan LJ, Fan XW, Yang HT, Wu JT, Wang SL, Qiu CG. MiR-93 inhibition ameliorates OGD/R induced cardiomyocyte apoptosis by targeting Nrf2. *Eur Rev Med Pharmacol Sci.* 2017;21(23):5456-5461.

24. Ke ZP, Xu P, Shi Y, Gao AM. MicroRNA-93 inhibits ischemia-reperfusion induced cardiomyocyte apoptosis by targeting PTEN. *Oncotarget.* 2016;7(20):28796-28805.

25. Wang H, Lu M, Yao M, Zhu W. Effects of treatment with an Hsp90 inhibitor in tumors based on 15 phase II clinical trials. *Mol Clin Oncol.* 2016;5(3):326-334.

26. Murphy BL, Obad S, Bihannic L, et al. Silencing of the miR-17-92 cluster family inhibits medulloblastoma progression. *Cancer Res.* 2013;73(23):7068-7078.

27. Smith AL, Iwanaga R, Drasin DJ, et al. The miR-106b-25 cluster targets Smad7, activates TGF-beta signaling, and induces EMT and tumor initiating cell characteristics downstream of Six1 in human breast cancer. *Oncogene.* 2012;31(50):5162-5171.

28. Udelson JE, Stevenson LW. The future of heart failure diagnosis, therapy, and management. *Circulation.* 2016;133(25):2671-2686.

29. Vaduganathan M, Butler J, Gheorghiade M. Transforming drug development in heart failure: navigating the regulatory crossroads. *Circ Heart Fail.* 2016;9(10):e003192.

How to cite this article: Guo J, Li S, Li Y, Yan C, Wan Q, Wang Z. HSP90 inhibitor 17-AAG prevents apoptosis of cardiomyocytes via miR-93-dependent mitigation of endoplasmic reticulum stress. *J Cell Biochem.* 2019;120:7888-7896. https://doi.org/10.1002/jcb.28064