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A Natural Model of Mouse Cardiac Myocyte Senescence

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Abstract

Many cardiac aging studies are performed on mice first and then, due to difficulty in mouse cardiomyocyte culture, applied the rat neonatal cardiomyocytes to further determine the mechanisms in vitro. Now, the technological challenge of mouse cardiomyocyte culture has been overcome and there is an increasing need for the senescence models of mouse cardiomyocytes. In this study, we have demonstrated that the senescence of mouse cardiomyocytes occurred with the extended culture time as shown by the increased β -galactosidase staining, increased p53 expression, decreased telomere activity, shortened telomere length, increased production of ROS, increased cell apoptosis, and impaired mitochondrial Ψ m. These senescent responses shared similar results in aged mouse heart tissues in vivo. In summary, we have established and characterized a novel senescence model of mouse cardiomyocytes induced by the extended culture time in vitro. The cell model could be useful for the increased cardiac aging studies worldwide.

Keywords

Cardiomyocyte; Cardiac aging; Mouse; Senescence; Cell model

The incidence of cardiac aging, a dominant risk factor for cardiovascular diseases, is increasing worldwide. Although the phenotypes of cardiac aging have been well characterized, the molecular mechanisms of cardiac aging are far from being fully elucidated.

Traditionally, many scientists performed the mouse studies of cardiac aging first in vivo. Then, they apply the rat neonatal cardiomyocytes to perform the senescence study in vitro [1]. In rat neonatal cardiomyocytes, senescence could be induced by ionizing radiation (IR), doxorubicin, hypoxia/reoxygenation, insulin-like growth factor 1 (IGF-1), and hydrogen per-

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Conflict of Interest The authors declare that they have no conflict of interest.

oxide (H₂O₂). Although these models of rat neonatal cardiomyocytes are excellent in verifying the molecular mechanisms of cardiac aging, there have a major weakness in this method: Many genes are different between rats and mice. For example, most of the thousands of long noncoding RNAs are not conserved between mice and rats.

In the past two decades, to uncover the molecular mechanisms of cardiac aging, many transgenic, gene knockout, pro-aging, and anti-aging mouse models have been generated, and the research of cardiac aging has been much improved with the help of these whole mouse models [1–3]. In contrast, the progress of senescence models of mouse cardiac myocytes in cultured cells in vitro is still very slow, although the cell models are critical for the mechanistic studies of cardiac aging. To date, only one senescence model of mouse cardiac myocytes has been established, in which the cell senescence is induced by doxorubicin [4]. In the current study, we wish to test whether or not the nature cultured mouse neonatal cardiomyocytes in vitro could induce senescence with increasing of the culture time, a phenomena identified in cultured rat neonatal cardiomyocytes? If the answer is yes, then, whether it could be used as a novel natural senescence model of mouse cardiac myocytes for cardiac study?

In this study, mouse cardiac myocytes were isolated from neonatal C57BL/6 mice (1 day old) and cultured with medium (76 % DMEM high glucose, 17 % F-12 medium, 4 % fetal bovine serum, 1 % penicillin/streptomycin, and 2 % 5 mM BrdU solution; the medium was changed every day) [5]. The cells were collected at 3 and 28 days after culture and then the cell senescence was evaluated by morphological analysis, senescence-associated β -galactosidase (β -gal) staining assay, intracellular reactive oxygen species (ROS), cell apoptosis, telomerase activity, telomere length, mitochondrial function, and the senescence-associated p53. We found that senescence occurred in mouse cardiomyocytes after 28 days of nature culture in vitro as demonstrated by the irregular large and flat shapes (Fig. 1a–c), increased senescence-associated β -gal staining (Fig. 1e–g), increased senescence-associated p53, decreased telomerase activity (Fig 1h), increased ROS production (Fig. 1i, j), increased apoptosis (Fig. 1k, l), and impaired mitochondrial function (Fig. 1m, n).

To test the potential application of this cell model in cardiac aging studies in vivo, we determined the senescent markers in aged mouse tissues. As shown in Fig. 5, compared with the heart tissues in young mice at 3 months old, senescence-associated β -gal staining (Fig. 1o–q) senescence-associated p53 (Fig. 1r, s), telomerase activity (Fig. 1t), telomere length (Fig. 1u), ROS production (Fig. 1v, w), mitochondrial Ψ m (Fig. 1x, y) in aged mice at 20 months age shared the similar significant changes with the natural senescent cardiomyocytes in vitro.

Senescence of mouse cardiomyocytes in hearts in vivo is a long and chronic process. However, we found for the first time that the senescence of mouse cardiomyocytes may occur quickly in naturally cultured cells in vitro. To date, the reasons why mouse cardiomyocytes could quickly change their phenotype to senescence are still unclear. We think that differences of their living environments could be at least an important reason. For example, in vivo, the hearts beat against a big pressure load, but it never occurs in cultured cardiomyocytes. In addition, the systemic neurohormonal regulations are lost in cultured

cardiac cells. The molecular mechanisms of nature culture-induced senescence mouse cardiomyocytes are currently unknown. We think it may be induced by multiple factors. We found in this study, at least, p53 may play a role in this senescent process.

The potential applications of this novel cell model in cardiac aging were further verified in vivo in aged mice. Indeed, we have found that the changes of multiple senescent makers including senescence-associated β -gal staining, senescence-associated p53, telomerase activity, telomere length, ROS production, and mitochondrial Ψ m in these senescent cardiomyocytes in vitro shared the similar significant changes with aged mouse hearts in vivo. In addition, these senescence changes are also similar to these reported in aged human hearts.

In summary, in this study, we have established and characterized a novel senescence model of mouse cardiac myocytes induced by the extended culture time in vitro. The novel cell model may have significant importance for the increasing cardiac aging studies worldwide.

Clinical relevance of the study

With the continuously growing elderly population, cardiac aging and its related heart disease is now a major health problem worldwide. In this study, we have created a natural senescence model of mouse cardiac myocytes, which have similar aging phenotypes to mouse cardiac aging and human cardiac aging in vivo and may have a significant clinical significance.

Acknowledgments

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Fig. 1.

A novel natural senescence model of mouse cardiac myocytes. Compared with the mouse cardiac myocytes at 3 days after culture, senescence occurred in cardiac myocytes at 28 days after culture as the irregular large and flat shapes (**a, b**) (*green color* α -actinin staining), increased senescence-associated β -gal staining (*blue color*) (**c–e**), increased senescence-associated p53 (**f**), decreased telomerase activity (**g**), reduced telomere length (**h**), increased ROS production (**i, j**), increased apoptosis (**k, l**), and impaired mitochondrial function (**m, n**). The impaired mitochondrial function was demonstrated by the decreased mitochondrial transmembrane potential (Ψ_m) levels (red/green intensity ratio). Note, $n = 6$; $*p < 0.05$ compared with that in cells cultured on day 3. Senescent markers of hearts from aged mice at 20 months old shared the similar significant changes with the natural senescent cardiomyocytes in vitro as shown by the increased β -gal staining (**o–q**), increased

senescence-associated p53 (**r**, **s**), decreased telomerase activity (**t**), reduced telomere length (**u**), increased ROS production (**v**, **w**), and decreased mitochondrial Ψ_m (**x**, **y**) compared with these in heart tissues from young mice at 3 months old. Note, $n = 6$; $*p < 0.05$ compared with that sections from young mice

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