Adenylyl Cyclase Type 5 Disruption Prolongs Longevity and Protects the Heart Against Stress

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Heart failure remains the leading cause of mortality in the USA, despite major advances in therapy over the past several decades, including angiotensin-converting enzyme or angiotensin II inhibitors, vasodilators, calcium-channel blockers and β-adrenergic receptor blockers. New therapeutic approaches are clearly required and the conceptual origin of these new techniques will be derived from agents that protect the heart against stress and prolong longevity. The combination of stress protection and longevity has been observed in a variety of organisms, from yeast to worms to mammals, and could be the basis for a novel approach to heart failure therapy. A mouse model has been developed with genetic disruption of adenylyl cyclase type 5, which lives one-third longer than the wild-type and is protected from aging-induced, pressure overload-induced and catecholamine-induced stresses. Accordingly, inhibition of this molecule should be considered as a new therapeutic modality for heart failure.


Key Words: β-adrenergic receptor signaling; Apoptosis; Cardioprotection; Pressure overload

It has been recognized for some time that aging and longevity are regulated by evolutionarily conserved molecular pathways, many of which also affect metabolism. Factors affecting basal metabolism (eg, growth hormone/insulin-like growth factor 1 (GH/IGF-1) signaling pathway or downstream effectors such as FOXO transcription factors) are all involved in the regulation of lifespan. Sir2, an NAD-dependent histone deacetylase, plays an essential role in mediating lifespan extension in diverse organisms; it is required for longevity because of caloric restriction in yeast and the fruit fly and elevated activity of Sir2 increases lifespan in yeast! Caenorhabditis (C.) elegans and the fruit fly. Although the direct effects of Sir2 on longevity need to be determined in mammals, mammalian Sir2 has shown favorable effects on longevity in mice. FOXO family transcription factors act as stress resistance factors that also control lifespan.

Interestingly, there is an interaction among these molecular mechanisms that regulate longevity; for example, in C. elegans, elevated SIR-2.1 expression leads to an increase in lifespan that is dependent on DAF-16, a FOXO transcription factor that is regulated by insulin signaling. Mutant mice with GH deficiency, including Ames, Snell dwarf mice and Little mice, all live longer and have delayed appearance of aging-associated phenotypes. A number of single gene mutations on the GH/IGF-1 pathway or its effectors, including GH receptor/binding protein knockout (GHR/BP−/−) IGF-1 receptor knockout (Igf1r−/−) mice, p66shc−/− mice, fat-specific insulin receptor knockout (FIRKO) mice and hormone Klotho overexpression mice, also extend lifespan significantly. The studies with these longevity models also noted the association with increased stress resistance, especially resistance to oxidative stress and apoptosis.

Our laboratory has discovered a novel molecular mechanism, residing in the β-adrenergic signaling pathway (ie, inhibition of adenylyl cyclase type 5). Adenylyl cyclase (AC) has 9 major mammalian isoforms, of which type 5 plays a major role in regulation of the heart and brain, as well as other organs. We have found that disruption of type 5 AC (AC5 KO) in the mouse prolongs lifespan and protects against stress similar to what has been described in the other genetic models of longevity described earlier. It is our concept that the inhibition of AC5 could become a novel therapy for heart failure and here we elaborate the basis for this concept, reviewing the data resulting in longevity and stress resistance in the AC5 KO mouse.

Longevity

In many normal wild-type (WT) mouse strains, as with 129SVJ used in the studies described here, 50% die by 24–26 months of age. Mice with disrupted AC5 live one-third longer than WT littermates (Fig 1). Kaplan-Meier statistics demonstrate not only that maximal survival is prolonged in AC5 KO, but also that the 50% survival point is extended from 26 months to 33 months, in both females and males.

Osteoporosis of Aging

We also observed that AC5 KO mice are protected from the osteoporosis of aging. Bone density at necropsy revealed weakened bones in WT mice, but preservation of bone integrity in old AC5 KO (Fig 1). Although this examination was conducted at autopsy, when bones in old mice are extremely fragile, measurements of bone strength and integrity...
Extended lifespan in adenylyl cyclase type 5 (AC5) knock-out (KO) mice. The Kaplan-Meier survival curve shows significantly increased survival (P<0.01) of AC5 KO mice compared with their wild-type (WT) littermates studied anterogradely from birth to death. Roughly 50% of WT mice died by 25 months, whereas 50% of AC5 KO mice died by 33 months. At 33 months, all WT mice had died, whereas 75% of AC5 KO mice were still alive. The maximum survival was also significantly different (P<0.02). (Reprinted with permission from Yan et al. Cell 2007; 130: 247–258.) Bone integrity was only studied in female mice. Radiographs taken of the tibia of aging WT and AC5 KO mice. The WT mice exhibit reduced bone density, apparent healing stress fractures, reduced calcification, and absence of the fibula. In contrast, bones appear normal in the radiographs of AC5 KO mice of the same age. (Reprinted with permission from Yan et al. Cell 2007; 130: 247–258.)

Adenylyl cyclase type 5 (AC5) knock-out (KO) mice are protected against aging cardiomyopathy. Comparison of left ventricular (LV) weight and body weight (A), LV ejection fraction (B), LV apoptosis (C) and LV fibrosis (D) in AC5 wild-type (WT) and KO mice, young (3–6 months, n=4–9) and old (20–30 months, n=4–9). There were no significant differences between young WT and young AC5 KO mice. However, all of the 4 parameters were significantly different in old WT compared with young WT (*P<0.05), characteristic of aging cardiomyopathy. In contrast, none of the 4 parameters was different in the old vs young AC5 KO, but all 4 parameters were significantly different in the old AC5 KO vs old WT (***P<0.05). (Reprinted with permission from Yan et al. Cell 2007; 130: 247–258.) *Old WT different from young WT, P<0.05. **Old AC5 KO different from old WT, P<0.05.
Longevity and Stress Resistance in AC5 KO

Cardiomyopathy of Aging

Old animals and humans exhibit a cardiomyopathy of aging (eg, the heart dilates and hypertrophies and there is increased apoptosis and fibrosis, which impairs cardiac function). We did observe increased myocyte hypertrophy, apoptosis and fibrosis and decreased cardiac function in the aging WT mice; however, none of these effects was observed in AC5 KO, in which normal cardiac function and architecture were preserved (Fig 2).23

Oxidative Stress

An important mechanism resulting in reduced longevity and susceptibility to stress is oxidative stress, and conversely protection against oxidative stress is salutary. We examined this in aging AC5 KO mice23 and found that the increased oxidative stress observed in old WT was not observed in old AC5 KO. For example, hydrogen peroxide and UV light can produce oxygen free radicals, which in turn, reduce cell viability and increase apoptosis. Using cell culture preparations for isolated cells, we found that these effects were significantly greater in myocytes from WT compared with AC5 KO. Thus, the AC5 KO is protected from oxidative stress.

Chronic Pressure Overload and Subsequent Development of Heart Failure

Chronic pressure overload results in hypertrophy and ultimately in increased wall stress with reduced cardiac function, progressing to heart failure. Chronic pressure overload was induced by banding the ascending aorta of the mice, resulting in a pressure gradient across the aortic constriction of roughly 100mmHg. We found that AC5 KO mice tolerated the pressure overload better than WT mice. Specifically, at 3 weeks after chronic aortic banding, LV function began to deteriorate in WT, but not in AC5 KO. Thus, WT mice progressed to heart failure more rapidly.
ly than AC5 KO, despite a similar degree of pressure overload.

**Chronic Catecholamine Stress**

It is well recognized that desensitization of the β-adrenergic receptor signaling pathway plays a critical role in the defense against heart failure (i.e., chronically enhanced signaling induced by stimulation of the sympathetic nervous system through the β-adrenergic receptor pathway is deleterious and results in enhanced metabolic demand secondary to increases in heart rate, cardiac contractility and wall stress, generally in situations of limited or reduced oxygen availability, e.g., in chronic coronary artery disease or dilated cardiomyopathy). Currently, inhibiting, as opposed to stimulating, the sympathetic nervous system and β-adrenergic receptor signaling is proving to be an important approach in treating heart failure. Because AC is central to β-adrenergic receptor signaling, inhibition of this molecule could be a new target for therapeutic intervention. Inhibiting the pathway at the level of AC5 could accomplish the same goal as blocking the β-adrenergic receptor, with less adverse effects of negative inotropy.

We tested this hypothesis in a recent study in which we...
Fig 7. Proposed mechanism mediating longevity and stress resistance in adenyl cyclase type 5 (AC5) knock-out (KO) mice. Knocking out AC5 activates the Raf/MEK/ERK signaling pathway. Activation of ERK activates anti-oxidative stress, anti-apoptosis and cell survival mechanisms, which lead to longevity in AC5 KO mice. The arrows indicate the direction of signaling (↑ increase, ↓ decrease). (Reprinted with permission from Yan et al. Cell 2007; 130: 247–258.)

delivered a high dose of isoproterenol for 1 week using an implanted mini osmotic pump. We found that AC5 KO were protected compared with WT and exhibited enhanced longevity in AC5 KO mice. This work was supported in part by NIH grants AG027211; HL031107; HL059139; HL069752; AG023137; AG014121.

References

23. Yan L, Vatner DE, O’Connor JP, Ivesa A, Ge H, Chen W, et al. Type 5 adenyl cyclase disruption increases longevity and protects longevity in AC5 KO (Fig 7). A number of other studies have demonstrated a protective role of the MEK-ERK pathway in mediating stress resistance.

In summary, our concept is that the combination of stress resistance and longevity is conserved from yeast to worms to mammals, and could be therefore a potentially important therapeutic approach for patients, particularly those with heart failure. One novel target is inhibition of type 5 AC. This concept is based upon the results from our studies of the AC5 KO mouse, which lives longer than WT mice and is protected from oxidative stress, chronic catecholamine stress and chronic pressure overload stress.

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