Molecular Cardiology

Apoptosis Signal-Regulating Kinase 1/p38 Signaling Pathway Negatively Regulates Physiological Hypertrophy

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Background—Mechanical stress on the heart can lead to crucially different outcomes. Physiological stimuli such as exercise cause adaptive cardiac hypertrophy, characterized by a normal cardiac structure and normal or enhanced cardiac function. Pathological stimuli such as hypertension and aortic valvular stenosis cause maladaptive cardiac remodeling and ultimately heart failure. Apoptosis signal-regulating kinase 1 (ASK1) is known to be involved in pathological cardiac remodeling, but it has not been determined whether ASK1 pathways coordinate the signaling cascade leading to physiological type cardiac growth.

Methods and Results—To evaluate the role of ASK1 in the physiological form of cardiac growth, mice lacking ASK1 (ASK1−/−) were exercised by swimming for 4 weeks. ASK1−/− mice showed exaggerated growth of the heart accompanied by typical characteristics of physiological hypertrophy. Their swimming-induced activation of Akt, a key molecule in the signaling cascade of physiological hypertrophy, increased more than that seen in wild-type controls. The activation of p38, a downstream kinase of ASK1, was suppressed selectively in the swimming-exercised ASK1−/− mice. Furthermore, the inhibition of ASK1 or p38 activity enhanced insulin-like growth factor 1–induced protein synthesis in rat neonatal ventricular cardiomyocytes, and the treatment with a specific inhibitor of p38 resulted in enhancement of Akt activation and suppression of protein phosphatase 2A activation. The cardiac-specific p38α-deficient mice developed an exacerbated form of cardiac hypertrophy in response to swimming exercise.

Conclusions—These results indicate that the ASK1/p38 signaling pathway negatively regulates physiological hypertrophy.

Key Words: exercise ■ hypertrophy ■ signal transduction

Mitogen-activated protein kinase pathways play a key role in mediating hypertrophy and apoptosis in cardiomyocytes.1 Apoptosis signal-regulating kinase 1 (ASK1) is a mitogen-activated protein kinase kinase that activates the c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinase and regulates cell death.2 In the heart, ASK1 is activated in response to various forms of stress such as pressure overload and neurohumoral factors that are known to mediate cardiac remodeling.3,4 Mice lacking ASK1 (ASK1−/−) showed less left ventricular (LV) remodeling and apoptotic cardiomyocytes 4 weeks after thoracic transverse aortic constriction or coronary artery ligation than did control wild-type (WT) mice, which suggests that ASK1 plays an important role in promoting LV remodeling and cell death.4 During our previous study of ASK1−/− mice,4 we noticed that the hearts of ASK1−/− mice were heavier when several mice were kept in 1 cage than when they were kept in individual cages. This led us to hypothesize that ASK1 might be involved in physiological hypertrophy.

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Physiological stimuli such as exercise result in cardiac hypertrophy, which is characterized by normal cardiac structure, preserved or improved cardiac function, and minimal alteration in the cardiac gene expression pattern.5 Physiological hypertrophy in well-trained athletes does not progress to heart failure,6,7 whereas pathological hypertrophy induced by pressure or volume overload is a strong predictor of heart failure.8 Recent studies have identified intracellular signaling pathways that play unique roles in the regulation of physiological cardiac hypertrophy,1,9 and among these, the phosphatidylinositol-3-kinase

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(PI3K)-Akt pathway has been proposed as a regulator of physiological growth of the heart. Cardiac-specific overexpression of activated PI3K(p110α) in transgenic mice resulted in baseline cardiac hypertrophy without fibrosis.10 Conversely, cardiac-specific overexpression of a dominant negative PI3K(p110α) mutant did not produce cardiac hypertrophy in response to exercise.11 PI3K generates phosphatidylinositol-3,4,5-trisphosphate (PIP3), a lipid second messenger essential for the translocation of Akt to the plasma membrane. Akt1-deficient mice are resistant to swimming exercise–induced cardiac hypertrophy.12

It is not yet clear whether the maladaptive pathological signaling pathway, which includes ASK1 and leads to heart failure, and the adaptive pathway leading to physiological hypertrophy are independent or interact with each other. In this study, we examined the role of ASK1 in the development of physiological hypertrophy.

Methods

**Animals**

This study was performed under the supervision of the Animal Research Committee in accordance with the Guidelines for Animal Experiments of Osaka University and the Japanese Government Animal Protection and Management Law (No. 105). The generation of ASK1−/− in the F2 generation on a C57Bl6/J background was described previously.13 Floxed p38α mice were crossed with α-myosin heavy chain promoter–driven Cre recombinase transgenic mice to obtain cardiac-specific p38α knockout mice (p38αCre−) and their littermate controls (p38αCre+).14 Mice were kept separate 4 weeks after birth and allowed access to water and mouse chow ad libitum.

**Swimming Exercise**

For long-term swimming exercise, groups of 8-week-old male mice were made to swim in water tanks twice daily for 90 minutes 7 days a week for 4 weeks.11 Before the swimming exercise, the mice practiced swimming for 8 days. The first day of swimming practice consisted of two 10-minute sessions separated by at least 4 hours. Sessions were then increased by 10 minutes each day until they reached the 90-minute target.

**In Vitro Kinase Assay, Phosphatase Assay, and Western Blot Analysis**

The activity of ASK1, Akt, and phosphoinositide-dependent kinase-1 (PDK1) was measured by an immature complex kinase assay as described.4,15,16 The protein phosphatase 2A (PP2A) activity was determined with the use of a Ser/Thr Phosphatase Assay System (Promega, Madison, Wis). Total protein homogenates (20 to 30 μg per lane) were subjected to Western blot analysis with antibodies against mouse p38 (C-20), JNK1 (FL), and ERK1 (K-23) obtained from Santa Cruz Biotechnology (Santa Cruz, Calif) and with antibodies against mouse p38, Akt, PI3K(p110), and phospho-Akt (Ser473) from Cell Signaling Technology Inc (Danvers, Mass).

**Assessment of Hypertrophic Responses in Vitro**

Neonatal rat ventricular cardiomyocytes (NRVM)13 were stimulated with insulin-like growth factor 1 (IGF-1), phenylephrine, or viral infection for 48 hours in the medium supplemented with [H]leucine.
Lipid Kinase Assay

Lipid kinase assays were performed as described elsewhere.10 The kinase reaction was performed at 37°C for 10 minutes, and lipids were extracted and analyzed by means of thin layer chromatography. After drying, the thin layer chromatography plates (Merck, Darmstadt, Germany) were subjected to autoradiography.

Statistical Analysis

Results are shown as mean±SEM. Paired data were evaluated by Student t test. A 1-way or 2-way ANOVA with the Bonferroni post hoc test was used for multiple comparisons. A value of P<0.05 was considered statistically significant.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Enhanced Cardiac Growth in ASK1−/− Mice as Response to Swimming Exercise

The ASK1−/− mice with a C57Bl6/J mouse background appeared normal and had a normal global cardiac structure and function compared with age-matched C57Bl6/J WT mice (Table), as we reported previously.4 WT and ASK1−/− mice were made to swim in 90-minute sessions twice daily for 4 weeks. We examined ASK1 activity in swimming-exercised WT hearts by an immune complex kinase assay using MKK6 as a substrate. We detected an 1.6-fold increase in ASK1 activity after 30 minutes of swimming (Figure 1A). Cardiac hypertrophy was assessed by measuring the ratio of LV weight to tibia length (LVW/tibia). There were no significant differences in LVW/tibia between control sedentary WT and ASK1−/− mice (WT; 4.83±0.07; ASK1−/−; 4.86±0.06 mg/mm) (Figure 1B). In response to swimming exercise, LVW/tibia increased significantly for both groups, and the degree of hypertrophy observed in ASK1−/− mice was significantly larger than that in WT controls (WT, 5.28±0.09; ASK1−/−; 5.78±0.07 mg/mm; P<0.05). There was no significant difference in the ratio of right ventricular weight to tibia length between swimming-exercised ASK1−/− and WT mice (data not shown). In humans, endurance training such as long-distance running and swimming increases ventricular chamber dimension in association with a mild increase or no change in wall thickness.13,14 The internal dimensions of LV at diastole in both WT and ASK1−/− mice were significantly elevated at the end of the swimming exercise program (Table), but the extent of LV dilatation was larger in ASK1−/− mice than in WT mice. The LV internal dimension at systole did not increase in either WT or ASK1−/− mice. Cardiac function, as indicated by fractional shortening, was enhanced in exercised WT and ASK1−/− mice compared with the sedentary mice. The diastolic interventricular septum thickness, LV posterior wall thickness, and LV mass significantly increased in exercised WT and ASK1−/− mice compared with the sedentary controls. The LV mass in WT mice was smaller than that of ASK1−/− mice after 4 weeks of swimming (Table). The cardiomyocyte cross-sectional area significantly increased in WT and ASK1−/− mice in response to the swimming program compared with that in sedentary controls (Figure 1C, 1D), and the extent of the
increase was significantly larger in ASK1−/− mice than in WT mice (WT, 256.8±3.6; ASK1−/−, 315.2±4.6 μm²; P<0.05). These results indicate that ASK1−/− mice developed a more profound eccentric type of cardiac hypertrophy than did WT after undergoing 4 weeks of exercise.

There was no fibrosis in the ventricular wall of swimming-exercised mice in either group (Figure 1E). Pressure overload by means of transverse aortic constriction, which is considered a pathological stimulus, was associated with increases in the mRNA expression levels of ANF and brain natriuretic peptide (Figure 1F). In contrast, the expression of these genes was not induced in the heart of swimming-exercised WT and ASK1−/− mice. These results indicate that the exercise-induced cardiac hypertrophy observed in ASK1−/− mice was physiological but not pathological.

Enhanced Akt Activation in Swimming-Exercised ASK1−/− Mice
Akt is required for physiological cardiac hypertrophy.12 We examined the level of Akt activation in swimming-exercised ASK1−/− hearts. We detected an ≈1.5-fold increase in the phosphorylation level of Akt after 30 minutes of swimming in WT hearts compared with the level in sedentary controls (Figure 2A). The phosphorylation level of Akt after 30 minutes of swimming in ASK1−/− mice was moderately but statistically significantly higher than in WT mice (Figure 2B). The Akt activity also was significantly higher than in WT mice (Figure 2C). These results indicate that ASK1 pathways may interact with the Akt signaling cascade.

Activation of p38 Selectively Inhibited in ASK1−/− Mice During Swimming
We next examined the activation profile of p38, a downstream kinase of ASK1, in WT and ASK1−/− hearts in response to swimming (Figure 2D). The mice were made to swim for 90 minutes and then rested for 6 hours. In the heart of WT mice, the phosphorylation level of p38 transiently increased after 30 minutes of swimming. The time course of p38 activation was similar to that of Akt. We then compared the activation pattern of p38 in WT and ASK1−/− mice during swimming and did not observe any significant p38 activation in ASK1−/− mice. On the other hand, WT and ASK1−/− mice showed slight and similar activation of JNK while swimming (data not shown).

Effect of p38 Inhibition on IGF-1-Induced Cardiomyocyte Hypertrophy In Vitro
Swimming exercise has been found to increase myocardial IGF-1 expression,19 and overexpression of IGF-1 or IGF-1 receptor in the heart has been found to induce physiological hypertrophy.20,21 IGF-1 treatment of NRVM is considered to be an in vitro model of physiological hypertrophy,22 and phenylephrine treatment of NRVM is considered to be a model of pathological hypertrophy.3 Incubation of NRVM with 100 μmol/L of phenylephrine produced an increase in [3H]leucine incorporation accompanied by the activation of the ANF promoter (Figure 3A). When NRVM were treated with IGF-1, we observed a significant increase in [3H]leucine incorporation in a dose-dependent manner without ANF promoter activation (Figure 3A). These findings indicate that treatment of NRVM with 10 to 100 nmol/L of IGF-1 mimics physiological hypertrophy under the conditions of our study. To confirm the role of ASK1 in physiological hypertrophy, we examined the effect of ASK1 inhibition on IGF-1-induced cardiomyocyte hypertrophy. To this end, we infected NRVM with an adenoviral vector expressing ASK(KR), a dominant negative mutant of ASK1 (Figure 3B). Overexpression of ASK(KR) enhanced IGF-1–induced [3H]leucine incorporation but did not ANF promoter activation.

We then examined the effect of p38 inhibition on IGF-1–induced cardiomyocyte hypertrophy in NRVM to identify the role of p38 in the enhanced physiological hypertrophy observed in ASK1−/− mice. Although pretreatment with SB203580, a specific inhibitor of p38, alone had no effect on [3H]leucine incorporation or ANF promoter activation, it enhanced IGF-1–induced cardiomyocyte hypertrophy without affecting ANF promoter activation (Figure 3C). Thus, the inhibition of p38 enhanced physiological hypertrophy. These results suggest that the ASK1/p38 signaling cascade negatively regulates physiological cardiac hypertrophy.
Effect of p38 Inhibition on IGF-1–Induced Akt and PI3K Activation

Because Akt plays a central role in physiological cardiac growth, we examined the relationship between p38 and Akt activation. The Akt phosphorylation by IGF-1 treatment reached a peak in 10 minutes and then declined in vehicle-pretreated NRVM (Figure 4A). In SB203580-pretreated NRVM, the phosphorylation of Akt was sustained up to 60 minutes after IGF-1 treatment, but it was significantly higher than that in vehicle-pretreated control 60 minutes after IGF-1 stimulation (Figure 4A).

We next examined the activation level of PI3K, which exists upstream of Akt in the signaling cascade leading to physiological hypertrophy. IGF-1 treatment significantly increased PI3K

Figure 3. Enhanced hypertrophic responses to IGF-1 by ASK1 or p38 inhibition in NRVM. Top, Incorporation of [3H]leucine was measured in NRVM stimulated with or without phenylephrine (100 μmol/L) or with IGF-1 for 48 hours in the presence of [3H]leucine (n=4). Bottom, ANF luciferase activity. Luciferase activity was measured in ANF-luciferase reporter construct-transfected NRVM stimulated with or without phenylephrine or with IGF-1 for 24 hours (n=3). Data are shown as fold increase relative to unstimulated controls. *P<0.05 (1-way ANOVA) vs unstimulated controls. †P<0.05 (1-way ANOVA) vs IGF-1–treated NRVM. A, IGF-1–induced cardiomyocyte hypertrophic responses. B, NRVM infected with adenoviral vector expressing ASK1(KR) or LacZ. Twenty-four hours after infection, NRVM were stimulated with 10 nmol/L IGF-1. C, NRVM were pretreated with 5 μmol/L SB203580 for 30 minutes and then stimulated with 10 nmol/L IGF-1.

Figure 4. Effect of p38 inhibition on PI3K/Akt signaling cascade. NRVM were pretreated with 5 μmol/L SB203580 (closed circles) or vehicle (open circles) for 30 minutes and were stimulated with IGF-1 for 60 minutes. NRVM pretreated with SB203580 were stimulated with IGF-1 for 60 minutes. Cell lysates were immunoprecipitated with anti-p110α–specific antibody and subjected to in vitro lipid kinase assay with the use of phosphatidylinositol as a substrate. PIP indicates phosphatidylinositol 3-phosphate. Bottom panel shows quantitative analysis. *P<0.05 vs any other groups (n=3). C, PDK1 activity. NRVM pretreated with SB203580 were stimulated with IGF-1 for 10, 30, and 60 minutes. PDK1 activity was measured by immune complex assay with the use of His-SGK1 as a substrate. Right panel shows quantitative data of 3 independent experiments. D, PP2A activity. NRVM pretreated with SB203580 were stimulated with IGF-1 for 10, 30, and 60 minutes. *P<0.05 (1-way ANOVA) vs vehicle-pretreated at corresponding time point (n=5). E, Akt phosphorylation. NRVM were pretreated with SB203580 and/or 1 μmol/L okadaic acid for 30 minutes and stimulated with IGF-1 for 60 minutes.
activity (Figure 4B), and pretreatment with SB203580 did not enhance IGF-1–induced PI3K activation but rather abolished the activation. Akt activity is regulated by phosphorylation and dephosphorylation.° PDK1 phosphorylates and activates Akt. The extent of the IGF-1–induced increase in PDK1 activity was similar between SB203580-pretreated and vehicle-pretreated NRVM at 10, 30, and 60 minutes after IGF-1 treatment (Figure 4C). The activity of PP2A, which dephosphorylates Akt, increased 10 minutes after IGF-1 treatment and was sustained thereafter, but pretreatment with SB203580 abolished the increase in the PP2A activity 60 minutes after IGF-1 treatment (Figure 4D). Furthermore, pretreatment with 1 μmol/L okadaic acid, an inhibitor of PP2A, resulted in an ∼1.2-fold increase in the phosphorylation level of Akt in NRVM treated with IGF-1 (n=3; *P<0.05) (Figure 4E). SB203580 had no effect on the phosphorylation level of Akt in IGF-1–treated, okadaic acid–pretreated NRVM. These results indicate that p38 may negatively regulate Akt activity through the activation of PP2A.

Cardiac-Specific p38α Knockout Mice Exhibited Enhanced Physiological Hypertrophy Induced by Swimming Exercise

To examine the in vivo role of p38 in swimming exercise–induced cardiac hypertrophy, cardiac-specific p38α knockout mice were made to swim. These mice were generated by crossing floxed p38α mice with α-myosin heavy chain promoter–driven Cre recombinase transgenic mice.° Physiological parameters such as body weight, heart weight, blood pressure, and heart rate as well as echocardiographic parameters such as LV internal dimension at diastole, LV internal dimension at systole, diastolic interventricular septum thickness, LV posterior wall thickness, and fractional shortening at baseline showed no differences between p38αCre− and their littermate controls, p38αCre− mice.° In response to the 4-week swimming program, LVW/tibia significantly increased in both p38αCre− and p38αCre− mice. However, the extent of hypertrophy was significantly larger in the former than in the latter (LVW/tibia for p38αCre− and p38αCre−, 5.44±0.16 and 6.15±0.16 mg/mm, respectively; *P<0.05) (Figure 5A). Swimming exercise significantly increased the cardiomyocyte cross-sectional area in p38αCre− and p38αCre− mice (Figure 5B, 5C), and the extent of the increase was significantly larger in p38αCre− mice than in p38αCre− mice (p38αCre−, 255.0±21.1; p38αCre−, 317.8±2.3 μm²; *P<0.05). There was no fibrosis in the ventricular wall of swimming-exercised mice in either group (Figure 5D). ANF and brain natriuretic peptide were not induced in p38αCre− and p38αCre− hearts at the end of the exercise program (Figure 5E). Cardiac function, as indicated by fractional shortening, was not changed by swimming exercise in p38αCre−, Cre− and p38αCre− mice (sedentary p38αCre−, 44.1±0.7%; p38αCre−, 43.9±0.8%; swimming-exercised p38αCre−, 44.4±0.5%; p38αCre−, 43.7±0.6%). The sedentary or swimming-exercised Cre transgenic mice showed no morphological or functional differences in the heart compared with corresponding nontransgenic littermate controls (Figure in the online-only Data Supplement). This excluded the possibility that toxicity of Cre protein contributed to the swimming exercise–induced phenotypes in p38αCre− mice. The phosphorylation level of Akt after 30 minutes of swimming in p38αCre− mice was moderately but statistically significantly higher than in p38αCre− mice (Figure 5F).

Discussion

The study presented here indicates that the AKT1/p38 signaling pathway negatively regulates physiological cardiac hy-
pertrophy. However, we cannot exclude the possibility that developmental, systemic, or other compensatory differences may contribute to the generation of the observed phenotypes. Nevertheless, our in vitro data showing that overexpression of a dominant negative mutant of ASK1 or p38 inhibitor treatment enhanced IGF-1–induced cardiomyocyte hypertrophy in NRVM render the negative role of ASK1/p38 in physiological hypertrophy plausible.

The IGF-1/P38/Akt signaling pathway plays a central role in the development of physiological hypertrophy in response to exercise.9,23 Here, we demonstrated that activation of Akt was enhanced by the inhibition of the ASK1/p38 cascade, indicating that cross talk occurs between the IGF-1/Akt and ASK1/p38 signaling pathways. Because we observed enhanced IGF-1–induced activation of Akt but not P38 in response to treatment with SB203580, the cross talk would be at the Akt level in the IGF-1/P38/Akt signaling cascade. The attenuation of IGF-1–induced P38 activation by the pretreatment of SB203580 will be due to a negative feedback to the activated Akt signaling pathway. It has been reported that the transgenic female mice with cardiac-specific expression of a dominant negative p38α developed more profound cardiac hypertrophy than did WT in response to pressure overload.24 The enhanced hypertrophy was associated with Akt activation and the inhibition of p38α-enhanced estrogen-induced Akt activation in NRVM. Thus, the cross talk between p38 and Akt plays an important role in the development of physiological hypertrophy as well as pathological hypertrophy.

The PIP3 production by P38 in response to various growth factors and neurohumoral factors and subsequent Akt phosphorylation are essential for the activation of Akt.9 PTPN11 (phosphatase and tensin homolog deleted on chromosome 10) protein regulates PIP3 levels and antagonizes P38/Akt signaling.25 The p38 has been reported to inhibit Akt activity mediated through the induction of PTPN11.26 Uregulation of PTPN11, however, does not appear to be involved in the cross talk between p38 and Akt in our system because p38 inhibition resulted in immediate enhancement of IGF-1–induced Akt activation in NRVM. Akt is phosphorylated by PKD1 and by a complex that contains the mammalian target of rapamycin (mTOR) and its associated protein, rictor.27 Activated Akt ultimately undergoes dephosphorylation of phosphatases such as PP2A and PH domain leucine-rich repeat protein phosphatase and returns to the inactive state.28 In this study, the treatment with SB203580 attenuated P2A activity 60 minutes after IGF-1 treatment, when IGF-1–induced Akt activation was declining after the peak. SB203580 did not affect PDK1 activity after IGF-1 treatment. Okadaic acid treatment enhanced IGF-1–induced Akt activation, but SB203580 did not further enhance its activation. Thus, p38 may negatively regulate Akt activity through the activation of P2A. This signal transduction mechanism has been reported to be involved in serum depletion–induced apoptosis.29 It has also reported that p38 positively regulates Akt activity during myogenesis.30 The regulation of Akt by p38 might depend on cell types or natures of stimuli. Various Akt binding proteins modulate its activation by external signals through interaction with different domains of the Akt protein.31 p38 may modulate such a complex network of regulatory proteins and pathways related to Akt activity.

A previous study has demonstrated that Akt has a dual adaptive function, one to promote physiological hypertrophy and the other to suppress pathological hypertrophy.12 It remains to be clarified, however, whether ASK1/p38 also has such a dual function in the 2 forms of hypertrophy because conflicting results have been reported regarding the role of ASK1 and p38 in pathological hypertrophy.13,32–34 The findings of our previous studies using ASK1– or p38–deficient mice indicate that these signaling molecules do not have a primary role in the development of pathological hypertrophy in response to pressure overload.4,14 It has been reported, however, that ASK1–/– mice showed reduced cardiac hypertrophy after angiotensin II infusion.32 It has even been proposed that ASK1 may play a prohypertrophic role in NRVM,34 in contrast to its antihypertrophic role observed under apparently similar experimental conditions.3 A study using transgenic mice expressing a dominant negative mutant of MKK3, MKK6, or p38α identified an antihypertrophic role for p38.33 Although the role of ASK1/p38 in pathological hypertrophy remains to be clarified, we can conclude here that the ASK1 signaling pathway, activated by pathological stimuli, leads to maladaptive cardiac remodeling and at the same time attenuates the physiological adaptive pathway involving the Akt signaling cascade. It has been reported that Akt phosphorylates and inhibits ASK1.35 In response to physiological or pathological external stresses, each of these downstream signaling cascades negatively regulates the other.

To summarize, we demonstrated for the first time that the ASK1/p38 signaling pathway negatively regulates physiological cardiac hypertrophy induced by swimming exercise. Inhibition of p38 promotes Akt activation in the development of physiological cardiac hypertrophy. These findings demonstrate the existence of an important mechanistic link between Akt and ASK1/p38 in physiological cardiac hypertrophy.

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Disclosures
None.

References
Exercise induces the physiological form of cardiac hypertrophy, which is a favorable adaptive response of the heart to increases in bodily demand. On the other hand, pathological hypertrophy is a maladaptive response to pathological stimuli such as pressure or volume overload and often progresses to heart failure. We have previously reported that the apoptosis signal-regulating kinase 1 (ASK1) is involved in the development of pathological cardiac remodeling. In this study, mice lacking ASK1 or its downstream mitogen-activated protein kinase, p38, showed exaggerated growth of the heart accompanied with increased Akt activity in response to swimming exercise. These results suggest that agents that inhibit ASK1 activation by pathological stimuli antagonize pathological cardiac remodeling while promoting physiological growth of the heart. Such agents could convert a maladaptive pathological type of hypertrophy to an adaptive physiological type and improve the function of diseased heart. It thus may be of benefit to selectively target ASK1 as a potential strategy for the treatment of patients with heart failure.
Apoptosis Signal-Regulating Kinase 1/p38 Signaling Pathway Negatively Regulates Physiological Hypertrophy

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Supplementary Figure 1. Cre expression itself has no effect on the cardiac phenotypes in response to swimming exercise. Eight-week-old Cre- and Cre+ mice were subjected to swimming exercise for 4 weeks. A, Left ventricular weight (LVW) (mg)/tibia length (tibia) (mm) ratios were obtained after resting (non-swim) or a 4-week swimming exercise program (swim). *P < 0.05 (2-way ANOVA) vs corresponding non-swim control. n = 3 for each group. B, Histological analysis of heart sections stained with hemotoxilin and eosin. Bars = 100 μm. C, Average myocyte cross-sectional areas from transverse cardiac sections. Cross-sectional areas of 100 cells per mouse were measured in random fields in three mice per group. *P < 0.05 (2-way ANOVA) vs corresponding non-swim control. D, Histological analysis of heart sections stained with Azan-Mallory. Bars = 100 μm. E, Echocardiographic parameters after swimming training. LVIDd, diastolic left ventricular internal dimension; LVIDs, systolic left ventricular internal dimension; FS, fractional shortening. n = 3 for each group. *P < 0.05 (2-way ANOVA) vs corresponding non-swim control.