Activation of the Bone Morphogenetic Protein Receptor by H11Kinase/Hsp22 Promotes Cardiac Cell Growth and Survival

Xiangzhen Sui, Dan Li, Hongyu Qiu, Vinciane Gaussin, Christophe Depre

Abstract—H11 kinase/Hsp22 (H11K) is a chaperone promoting cardiac cell growth and survival through the activation of Akt, a downstream effector of phosphatidylinositol 3-kinase (PI3K). In this study, we tested whether H11K-induced activation of the PI3K/Akt pathway is mediated by the bone morphogenetic protein (BMP) signaling, both in a transgenic mouse model with cardiac-specific overexpression of H11K and in isolated cardiac myocytes. Microarrays in hearts from transgenic compared to wild-type mice showed an upregulation of the BMP receptors Alk3 and BMPR-II, and of their ligand BMP4 (P<0.01 versus wild type). Activation of the BMP pathway in transgenic mice was confirmed by increased phosphorylation of the “canonical” BMP effectors Smad 1/5/8 (P<0.01 versus wild type). In isolated myocytes, adenovirus-mediated overexpression of H11K was accompanied by a significant (P<0.01) increase in PI3K activity, phospho-Akt, Smad 1/5/8 phosphorylation and [3H]phenylalanine incorporation, and by a 70% reduction in H2O2-mediated apoptosis. All these effects were abolished by the BMP antagonist noggin. In presence of BMP4, Smad 1/5/8 phosphorylation was enhanced by 5-fold on H11K overexpression but decreased by 3-fold on H11K knockdown (P<0.01 versus control), showing that H11K potentiates the BMP signaling. In pull-down experiments, H11K increased both the association of Alk3 and BMPR-II together, and their interaction with the transforming growth factor-β (TGF-β) receptor type II (TGF-βR-II), a “noncanonical” mediator of the BMP receptor signaling. TAK1 inhibition prevented H11K-mediated activation of Akt. Therefore, potentiation of the BMP receptor by H11K promotes an activation of the PI3K/Akt pathway mediated by TAK1, which dictates the physiological effects of H11K on cardiac cell growth and survival. (Circ Res. 2009;104:00-00.)

Key Words: Akt ■ bone morphogenetic protein ■ heat shock protein ■ phosphatidylinositol 3-kinase

H11 kinase/Hsp22 (H11K), which belongs to the crystallin family of heat shock proteins, is mainly expressed in heart and skeletal muscle.1 An increase in H11K expression was found in a canine model of left ventricular hypertrophy,2 in swine models of acute and repetitive myocardial ischemia,3,4 and in patients with ischemic heart disease.5 We generated a transgenic (TG) mouse with cardiac-specific overexpression of H11K and in isolated cardiac myocytes. Microarrays in hearts from transgenic compared to wild-type mice showed an upregulation of the BMP receptors Alk3 and BMPR-II, and of their ligand BMP4 (P<0.01 versus wild type).10 In isolated myocytes, adenovirus-mediated overexpression of H11K was accompanied by a significant (P<0.01) increase in PI3K activity, phospho-Akt, Smad 1/5/8 phosphorylation and [3H]phenylalanine incorporation, and by a 70% reduction in H2O2-mediated apoptosis. All these effects were abolished by the BMP antagonist noggin. In presence of BMP4, Smad 1/5/8 phosphorylation was enhanced by 5-fold on H11K overexpression but decreased by 3-fold on H11K knockdown (P<0.01 versus control), showing that H11K potentiates the BMP signaling. In pull-down experiments, H11K increased both the association of Alk3 and BMPR-II together, and their interaction with the transforming growth factor-β (TGF-β) receptor type II (TGF-βR-II), a “noncanonical” mediator of the BMP receptor signaling. TAK1 inhibition prevented H11K-mediated activation of Akt. Therefore, potentiation of the BMP receptor by H11K promotes an activation of the PI3K/Akt pathway mediated by TAK1, which dictates the physiological effects of H11K on cardiac cell growth and survival.
Figure 1. Activation of the PI3K pathway in the TG mouse. A, Expression and phosphorylation of the p85 subunit, and activity of PI3K (determined by the detection of phosphatidylinositol trisphosphate,PIP3) in total protein extracts from TG mice vs WT (n=3/group). B, Immunoblotting for PDK1 and Akt in plasma membrane from TG vs WT (n=3/group). The α2 Na+/K+ ATPase (Na/K) is a normalizer. C, IGF receptor (IGF-R), phospho-IGF-R, BMP4, phospho-Smad 1/5/8 (P-Smad), and α-adrenergic receptor (α-AR) expression in TG vs WT (n=4/group). Glucose-6-phosphate dehydrogenase (G6-PDH) is a normalizer. "P<0.01 vs WT.

Materials and Methods

Animal Model

Three-month-old male TG mice with cardiac-specific overexpression of the hemagglutinin-tagged human H11K and their wild-type (WT) littermates were used, as characterized previously.2,5,13

Protein Extraction

Proteins were extracted and centrifuged at 12,000 × g for 20 minutes at 4°C in a buffer supplemented with protease and phosphatase inhibitors. Subcellular fractions were prepared in hypotonic buffer as before.13 Immuno precipitation was performed using 30 µg of PBS-washed protein A–sepharose incubated overnight at 4°C with 1 µg of antibody, followed by addition of 100 µg of cellular extract for 2 hours, after which the complex was washed 3 times.3 Proteins were denatured, resolved on SDS-polyacrylamide gels and transferred to nitrocellulose membranes. Immunoblotting were performed with the following antibodies (all from Cell Signaling, Danvers, Mass): p85 subunit of PI3K, phospho-p85, PDK1, Akt, phospho-Akt (both T308 and S473), insulin-like growth factor (IGF) receptor (total protein and phosphoprotein), BMP4, Smad5, phospho-Smad 1/5/8, α-adrenergic receptor, BMPR-II, Alk3, TAK1, p38 mitogen-activated protein kinase (MAPK) (total protein and phosphoprotein), and glucose-6-phosphate dehydrogenase. The antibody recognizing the α2 subunit of Na+/K+ ATPase was from Santa Cruz Biotechnology (Santa Cruz, Calif). The H11K antibody was used as before.2 The H11K and p85 antibodies were used at 1/500 dilution. All other antibodies were used at 1/500 dilution. After incubation with the secondary antibody, the signal was detected by enhanced chemiluminescence (Dupont/NEN, Boston, Mass) and quantified by densitometry.

PI3K Activity

The immunoprecipitated p85 subunit of PI3K was added to a buffer (50 mmol/L Tris pH 7.4, 100 mmol/L MgCl2, 1 mmol/L EDTA) supplemented with phosphatidylinositol and phosphatidyserine. The reaction was started by adding a solution containing [γ-32P]ATP (~3000 Ci/mmol) and conducted at 25°C for 20 minutes. Lipids were extracted in CHCl3/methanol (1:1) and applied on silica plates (60 K6F) coated with 1% potassium oxalate. Migration was performed during 30 minutes on soaking of the plate base with CHCl3/methanol/ammonia/water (45:35:1.5:8.5), followed by over-night exposure to a radiosensitive film.

cDNA Microarrays

Messenger RNA was used for first strand cDNA synthesis with the SuperScript reverse transcriptase and a T7-oligo(24)IT primer. Second-strand synthesis was performed with DNA polymerase I, subsequently transcribed into biotin-labeled synthetic antisense RNA (Bioarray RNA Labeling, ENZO, New York, NY), and hybridized to GeneChip 430.2.0 (Affymetrix, Santa Clara, Calif). Data were analyzed with the Welch’s t test, which assumes independent variation, with the Benjamin–Hochberg method, which adjusts probability values with the overall false discovery rate, and with the Significance Analysis of Microarray. Significance was determined by a P<0.05.

Cell Culture

Cardiac myocytes were cultured from 1-day-old rats as before.2 The adenovirus harboring H11K was described before.2 An adenovirus harboring β-Gal was used as a control. All experiments were performed 48 hours after infection in serum-free medium. Noggin and BMP4 (both from R&D Biosystems, Minneapolis, Minn) were added to the medium for 24 hours. Wortmannin (Sigma, St Louis, Mo) and 5Z-7-oxozeaenol (Analyticon, Potsdam, Germany) were added overnight. Concentrations are indicated in the figures and figure legends. Protein synthesis was measured by [3H]phenylalanine incorporation.14 Apoptosis was measured by caspase-3 activity15 and by TUNEL staining, which was performed on sections treated with 2% H2O2 to inactivate peroxidases and with 20 µg/mL proteinase K for permeabilization. DNA fragments were labeled with 2 nmol/L biotinylated dUTP and 0.1 U/µL deoxyribonuclease transferase. Incorporation of biotin-16-dUTP was measured with FITC-ExtrAvidin (Sigma Aldrich, St Louis, Mo). Slides were mounted for fluorescent microscopic observation at X40 objective field. Nuclear counterstaining was performed with DAPI. TUNEL-positive and DAPI-positive cells were counted on at least ten different fields. The number of apoptotic cells was calculated as a ratio of TUNEL/DAPI.
**H11K Silencing**

The U6 RNA polymerase III-dependent promoter and the polycloning region of the pSilencer 1.0-U6 expression vector (Ambion, Austin, Tex) were subcloned into the adenoviral shuttle vector pDC311. A short hairpin RNA was designed from the mouse H11K sequence (TTCAACAACGAGCTTCCTCATTCAAGAGAAATGGAGGAAGCTCGTTGAATTTTT), extended with ApaI- and HindIII-compatible overhangs, annealed, and subcloned distal to the U6 promoter. A recombinant adenovirus was generated in 293 cells using homologous recombination between the hairpin and the pBHGloxH9004E1,E3Cre vector (Microbix, Ontario, Canada).

**Statistical Analysis**

Results are the means±SEM for the number of samples indicated in the figure legends. Comparison was performed using the Student’s t test. Two-way ANOVA with Fisher correction was performed for multigroup comparison. A value of *P*<0.05 was considered significant.

**Results**

**Activation of the PI3K Pathway by H11K**

We previously described that H11K overexpression increases Akt phosphorylation in vivo. We tested whether this activation originates from an increased activity of PI3K, the upstream activator of Akt. Protein expression and the activity of PI3K were measured in total protein homogenates from WT and TG hearts. Although the expression of the regulatory p85 subunit of PI3K was comparable between both groups, both p85 phosphorylation (Y548) and PI3K activity were significantly increased by 3-fold in TG mice compared to WT (Figure 1A). Activation of the PI3K pathway was confirmed by the translocation to the plasma membrane of the PI3K effectors, PDK1 and Akt (Figure 1B). This translocation is accompanied by an increased phosphorylation of Akt on the PDK1-dependent site (T308). These results confirmed the activation of the PI3K signaling in the TG mouse.

Microarrays were performed in hearts from TG and WT mice to determine the transcriptional regulation of growth factors and receptors that could explain the H11K-mediated activation of PI3K. Surprisingly, the majority of growth factor receptors (tyrosine kinases or G-coupled receptors), such as the β-adrenergic receptor for example, were downregulated in the TG mouse compared to WT (Table). The only genes showing an upregulation encoded the IGF1 receptor (IGF-R), the BMP receptors Alk3 and BMPR-II, as well as one of the BMP receptor ligands, BMP4 (Table). These results were validated by western blotting. The expression of IGF-R protein was significantly increased by 3-fold in TG versus WT (Figure 1C). However, the phosphorylation of the receptor was decreased by 60% (*P*<0.05) in the TG (Figure 1C), suggesting that, even if IGF-R expression is increased, its specific activity might not be affected. Similarly, BMP4 expression was also significantly increased by 3-fold in TG versus WT (Figure 1C). To confirm that increased BMP4 expression resulted in the actual activation of the BMP pathway, the phosphorylation of the canonical BMP effectors, Smad 1/5/8, was measured using an antibody.

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recognizing all three phospho-isoforms. Phosphorylation of Smad 1/5/8 was more than doubled (\(P < 0.05\)) in TG versus WT (Figure 1C). Reciprocally, the \(\beta\)-adrenergic receptor showed a 75% reduction in TG hearts compared to WT (Figure 1C), in agreement with the microarray data (Table). The complete list of regulated genes is provided in Table I in the online data supplement, available at http://circres.ahajournals.org.

**BMP Is Necessary for H11K-Mediated Activation of PI3K**

These findings in heart homogenates in vivo were investigated more mechanistically on adenovirus-mediated overexpression of H11K in isolated cardiac myocytes infected for 48 hours with 5 multiples of infection (mois) of an adenovirus harboring the H11K sequence. Compared to the \(\beta\)-Gal control, the H11K adenovirus induced a 3-fold increase in H11K expression, which was accompanied by a 2-fold increase in PI3K activity and Akt phosphorylation, and by a 2-fold increase in Smad 1/5/8 phosphorylation (Figure 2A). In a dose–response experiment, the phosphorylation of Smad 1/5/8 increased proportionately to the amount of adenovirus (Figure 2B). Therefore, short-term overexpression of H11K reproduces the activation of both PI3K/Akt and BMP pathways, as found in the TG mouse.

We determined next whether inhibition of the BMP signaling would prevent the activation of the PI3K/Akt pathway by H11K. The BMP receptor can be inhibited by noggin, which blocks the interaction between the BMP ligands and their receptors.\(^6\) In isolated cardiomyocytes, the phosphorylation of Smad 1/5/8 on overexpression of H11K was abolished by noggin (Figure 2B). In addition, H11K-mediated activation of PI3K and increased Akt phosphorylation on both sites (T308 and S473) were also suppressed by noggin (Figure 2C and 2D), indicating that H11K-mediated activation of the PI3K/Akt pathway depends on the activity of the BMP receptor.

Because H11K is both a chaperone and a kinase,\(^6\) this experiment was repeated with an adenovirus harboring the sequence of a kinase-dead mutant of H11K (H11-KI).\(^17\) In that condition, H11K overexpression still increased Smad phosphorylation (Figure 2E). We showed before that H11-KI also activates the PI3K/Akt pathway,\(^17\) indicating that the activation of both BMP and PI3K/Akt pathways is performed by the chaperone function, rather than by the kinase function, of H11K.

**H11K Potentiates the BMP Signaling Pathway**

To delineate the mechanisms by which H11K activates the BMP pathway, we examined the effects of H11K overexpression on the phosphorylation of Smad 1/5/8 in presence of increasing concentrations of BMP4. In cells infected with \(\beta\)-Gal, phosphorylation of Smad 1/5/8 increased by 4- to 5-fold at the lowest dose of BMP4 tested (10 ng/mL) and it did not show any further increase at higher doses (Figure 3A). The same experiment was repeated on overexpression of H11K. In that case, Smad phosphorylation was significantly increased when compared to the same doses of BMP4 in absence of H11K (Figure 3). In addition, Smad phosphorylation in presence of H11K increased proportionately to the dose of BMP4, in contrast to the plateau observed in presence of the \(\beta\)-Gal control (Figure 3A). This experiment shows that H11K overexpression is sufficient to amplify the BMP receptor activity.

In a reciprocal experiment, we tested whether H11K expression is also necessary for BMP signaling. H11K knockdown was performed in cardiac myocytes using adenovirus-mediated delivery of a short hairpin RNA targeting the H11K sequence. A
hairpin targeting luciferase was used as a control. In the presence of the luciferase control, addition of BMP4 to the myocytes significantly increased H11K expression, as well as the phosphorylation of Smad 1/5/8 and Akt (Figure 3B). These measurements were repeated on H11K silencing. Compared to the luciferase control, addition of the adenovirus silencing H11K significantly reduced H11K protein abundance in absence of BMP4 and totally prevented the BMP4-mediated increase in H11K protein expression (Figure 3B). In addition, H11K knock-down reduced by 40% Smad 1/5/8 phosphorylation in absence of BMP4 (P<0.01), and suppressed the BMP4-mediated increase in Smad 1/5/8 phosphorylation found in control conditions (Figure 3B). BMP4-mediated increase in Akt phosphorylation was also suppressed on H11K knockdown (Figure 3B). Therefore, H11K is necessary for proper BMP signaling in cardiac cells.

**H11KCoprecipitates With the BMP Receptor**

To determine the mechanism by which H11K activates the BMP receptor, we tested whether H11K either quantitatively increases the abundance of BMP receptors on the plasma membrane, or whether it qualitatively increases the intrinsic activity of these receptors. To test the first possibility, membrane fractions were isolated from hearts of WT and TG hearts. The abundance of Alk3 and BMPR-II was determined by western blot in these fractions but it did not show any significant difference between both groups (Figure 4A).

To test the second possibility, ie, a qualitative effect on the BMP receptors, we determined whether H11K interacts with these receptors. Pull-down of either Alk3 or BMPR-II and subsequent western blot for H11K demonstrated that H11K coprecipitates with both receptors (Figure 4B). Coprecipitation of H11K with the BMP receptors was more abundant in the TG compared to WT, although an equivalent amount of receptor protein was pulled down (Figure 4B). In addition, the interaction between Alk3 and BMPR-II was more pronounced in TG compared to WT (Figure 4B).

The reciprocal experiment was performed in isolated cardiac myocytes infected with the adenovirus harboring the hairpin silencing H11K. In that condition, pull-down of either Alk3 or BMPR-II and subsequent western blot for the reciprocal protein showed that H11K knockdown dramatically decreases the coprecipitation of Alk3 with BMPR-II (Figure 4C). These data indicate that H11K interaction with Alk3 and BMPR-II promotes the association of these components into a functional receptor complex, which may explain the increase in BMP receptor activity on H11K overexpression.

**BMP-Mediated Activation of PI3K Involves TAK1**

The experiments presented above show that H11K-mediated stimulation of the BMP receptor leads to the activation of PI3K/Akt. We investigated which part of the BMP signaling triggers such activation. Although we relied on Smad 1/5/8 phosphorylation to demonstrate the activation or inhibition of the BMP receptor in our different experimental conditions, it is unlikely that the canonical Smad pathway is responsible for PI3K activation because Smad proteins are first and foremost transcription factors. Two experimental evidences made us
hypothesize that the “noncanonical” TAK1 pathway represents the molecular link between the BMP receptor and PI3K/Akt. The first evidence is that TAK1 interacts with H11K in melanoma cells.18 Second, TAK1 paradoxically promotes cell survival in osteoclasts through activation of Akt19 but also apoptosis in melanoma cells through activation of p38 MAPK.18 This paradox reflects the dual properties of H11K, which promotes survival at physiological concentrations in vivo but promotes apoptosis on excessive overload in vitro.17 Therefore, we determined whether TAK1 interacts with H11K in the heart, and whether TAK1 affects p38 MAPK and Akt activities in our model.

The interaction of TAK1 with the BMP receptors and with H11K was tested in vivo. Pull-down of TAK1 from cardiac extracts of WT and TG mice was accompanied by a coprecipitation of both Alk3 and BMPR-II, as well as of H11K (Figure 4D). In each case, the signal was stronger in samples from TG mice compared to WT (Figure 4D), showing that H11K promotes not only the formation of the BMP receptor complex (Figure 4B and 4C) but also the association of this complex with the TAK1 effector.

Because of the dual effect of both TAK1 and H11K on cell survival and apoptosis, we measured the dose-dependent consequences of H11K overexpression on the phosphorylation of TAK1, p38 MAPK and Akt. Cardiac myocytes were infected with doses of H11K adenovirus ranging from 5 to 20 moi. Phosphorylation of TAK1 on S412 increased at low doses of H11K and decreased thereafter, whereas p38 MAPK phosphorylation on T180/Y182 increased only at the highest dose (Figure 5A). A decreased phosphorylation of TAK1 on p38 MAPK activation may be explained by the previously described negative feed-back of p38 MAPK on TAK1 phosphorylation.20 Phosphorylation of Akt on the PDK1-dependent site (T308) showed a pattern similar to that of TAK1, ie, increased phosphorylation at low doses of H11K and decreased phosphorylation thereafter (Figure 5A), whereas increased apoptosis, measured by TUNEL staining, followed the pattern of p38 MAPK activation (Figure 5A). Therefore, low doses of H11K activate TAK1 and Akt, whereas higher doses activate p38 MAPK and apoptosis. H11K-mediated phosphorylation of TAK1 was abolished by noggin, confirming that such activation is controlled by the BMP receptor (Figure 5B).

The similar phosphorylation pattern of TAK1 and Akt suggests that TAK1 could be the link between the BMP receptor and the PI3K/Akt pathway. To test that possibility, cardiac myocytes were infected with the H11K adenovirus in presence of different concentrations of 5Z-7-oxozeaenol, a TAK1 inhibitor.21 As shown in Figure 5c, the inhibitor dose dependently abolished H11K-mediated increase in Akt phosphorylation on the PDK1-dependent site (T308), demonstrating that H11K-mediated stimulation of the BMP receptor activates the PI3K/Akt pathway through TAK1 (Figure 5D).

**The BMP Receptor/PI3K Pathway Mediates the Physiological Effects H11K**

We showed before that increased expression of H11K in the heart promotes cardiac cell growth2,13 and survival.3,5 We
tested whether the BMP pathway is necessary for such effects. To test whether the BMP pathway is necessary for the growth by H11K, cardiac myocytes were infected with an adenovirus harboring the β-Gal control or the H11K sequence, in presence or in absence of noggin, and the protein synthesis rate was measured by the incorporation of [3H]phenylalanine. Overexpression of H11K significantly increased protein synthesis when compared to the β-Gal control, which was totally suppressed by noggin (Figure 6A).

To test whether the BMP pathway is also necessary for the survival by H11K, apoptosis was induced by addition of H2O2 in presence of the adenovirus harboring β-Gal or H11K. Measurement of caspase-3 activity showed a significant 3-fold increase after addition of H2O2 in presence of the β-Gal control, and such increase was reduced by 70% on overexpression of H11K (Figure 6B). Addition of noggin in absence of H2O2 did not affect apoptosis in either group (Figure 6B). However, addition of noggin together with H2O2 significantly increased caspase-3 activity by ≈4-fold in presence of β-Gal, and abolished the protective effect of H11K (Figure 6B). Comparable results were obtained when measuring apoptosis by TUNEL (Figure 6C).

We measured whether PI3K is the downstream effector determining the survival effect of H11K. Cardiac myocytes, infected with the adenovirus harboring H11K or the LacZ control, were incubated with 1 μmol/L of the PI3K inhibitor wortmannin, in presence or in absence of H2O2, and apoptosis was measured by TUNEL. Wortmannin abolished the protective effects of H11K against apoptosis (Figure 6D). We showed before that inhibition of PI3K also blocks the growth-promoting effect of H11K. Therefore, an active BMP/PI3K axis dictates the physiological effects of H11K on cardiac cell growth and survival.

**Discussion**

We show that H11K promotes the formation of the BMP receptor complex and its association with the effector TAK1, which mediates the activation of the PI3K/Akt pathway responsible for the physiological effects of H11K on cardiac cell growth and survival. This conclusion is supported by the following observations. First, H11K-mediated activation of the PI3K signaling depends on an active BMP receptor complex. Second, H11K is required for proper BMP signaling in cardiac myocytes. Third, TAK1 represents the molec-
ular link between the BMP receptor complex and the PI3K pathway. Fourth, BMP is necessary for the physiological effects of H11K on cardiac cell growth and survival. Fifth, such effects of BMP are mediated by PI3K. Taken together, these data offer a novel mechanism for the cytoprotective effects of H11K and expand the role of the BMP signaling in postnatal heart.

H11K overexpression increases the formation of the BMP receptor complex. Yeast 2-hybrid experiments have shown that BMPR-II interacts with multiple signaling proteins, including PKC\(_\beta\), the protein tyrosine kinase 9, a protooncogene serine/threonine kinase, and MAPKKK8, but also with the heat shock protein B-crystallin.\(^2^2\) H11K is structurally close to B-crystallin, as both proteins share a similar crystallin domain in their C terminus,\(^2^3\) and both proteins interact.\(^2^4\) In addition, the heat shock protein Hsp27, which also interacts with H11K,\(^2^5\) promotes cardiac cell survival through a PI3K-dependent pathway, although the mechanism by which Hsp27 activates PI3K is not elucidated.\(^2^6\) Our observations depend on the chaperone function, rather than the kinase function, of H11K. Therefore, it is possible that a network of heat shock proteins interacts to promote cardiac cell growth and survival by maintaining the interaction between BMP and PI3K pathways. This mechanism would be complementary to other antiapoptotic actions of heat shock proteins.\(^2^7\)

The interaction of H11K with the BMP pathway explains the paradox that H11K is cytoprotective at low expression levels in vivo, and proapoptotic at supraphysiological concentration (or “overload”) in vitro.\(^6\) The cytoprotective effects, mediated by the PI3K/Akt pathway, are observed when H11K protein expression increases by 3- to 5-fold, which is found in vivo across species, such as dog, swine and humans, in both conditions of myocardial growth and ischemia,\(^4\) and in our TG model.\(^2,^5\) Reciprocally, proapoptotic effects occur in cell culture through the activation of p38 MAPK when H11K is excessively overloaded. We show that the prosurvival versus prodeath effects of H11K crucially depend on the balance between TAK1, p38 MAPK and Akt activity.

The microarrays show a specific upregulation of the BMP receptors Alk3 and BMPR-II, whereas most other growth factor receptors are downregulated. This downregulation includes proangiogenic growth factors, although it is not known whether this translates into impaired angiogenesis because the BMP pathway itself promotes angiogenesis.\(^2^8\) Knockout models have shown the essential role played by both BMP2 and BMP4 in cardiac development.\(^2^9,^3^0\) However, few studies have addressed the potential role of BMP in postnatal heart. In cardiac myocytes, activation of Smad 1 by BMP2 inhibits apoptosis.\(^3^1,^3^2\) In that model, BMP2 stimulates PI3K activity,\(^3^3\) but the molecular mechanisms involved were not explored. These studies relied on BMP2 to activate the BMP receptor, whereas we used BMP4 based on our microarray data confirmed by immunoblotting. Potential differences between cardiac stimulation by BMP2 or by BMP4 have not been investigated. How BMP4 transcript increases in our model is not elucidated. PI3K activates the transcription of BMP2 by MEF-2A,\(^3^1\) but it is not known whether PI3K may have a similar effect on BMP4 expression.

In conclusion, this study offers a more mechanistic insight into the cardioprotective effects of H11K and sheds more
light on the role of BMP in postnatal heart. Promoting the crosstalk between BMP and PI3K may represent a novel approach to prevent cell death in a context of cardiac stress.

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Disclosures
None.

References


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