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MEK1-Induced Physiological Hypertrophy Inhibits Chronic Post-Myocardial Infarction Remodeling in Mice

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ABSTRACT

Although activation of MEK-ERK signaling is known to be cardioprotective during acute reperfusion injury, the effect of MEK activation on chronic changes in ventricular structure and function during the more complex process of remodeling after myocardial infarction (MI) with or without reperfusion remains uncertain. Four weeks after permanent coronary ligation, LV fractional shorting, preload recruitable stroke work, and end-systolic elastance were all preserved in transgenic mice with CM-specific upregulation of the MEK1-ERK1/2 signaling pathway (MEK1 Tg) compared to wildtype (WT) controls (5.8% decline vs. 17.3%, $P < 0.01$; 603 ± 98 mmHg vs. 335 ± 98, $P < 0.05$; 6.14 ± 0.57 mmHg/μl vs. 3.92 ± 0.60, $P < 0.05$, respectively). Despite similar initial infarct sizes, post-MI remodeling was significantly reduced in MEK1 Tg, demonstrated by reductions in chronic infarct size ($28.5 \pm 3.1\%$ vs. $47.8 \pm 3.2\%$), myocardial fibrosis ($3.98 \pm 0.74\%$ vs. $9.27 \pm 1.97\%$) and apoptosis ($0.66 \pm 0.11\%$ vs. $1.60 \pm 0.34\%$). Higher phosphorylation (i.e., activation) of pro-survival transcription factor STAT3, higher expression of anti-apoptotic protein Bcl2, and higher phosphorylation (i.e., inactivation) of pro-apoptotic BAD were observed in the post-MI remote myocardium of MEK1 Tg. MMP2 activity was higher in MEK1 Tg, while expression of TIMP3 and MMP9 activity were lower in transgenic mice. Beyond any immediate cardioprotective effect, therapeutic activation of MEK1-ERK1/2 signaling during the chronic post-MI period may preserve LV function by increasing the expression of pro-survival factors and by suppressing factors, such as the balance between matrix modulating proteins, that promote pathological remodeling in the remote myocardium. J. Cell. Biochem. 114: 47–55, 2013.

KEY WORDS: MYOCARDIAL INFARCTION; HYPERTROPHY; MAP KINASE; REMODELING; MATRIX METALLOPROTEINASE; APOPTOSIS

The majority of morbidity and mortality associated with post-MI cardiomyopathy and heart failure results from chronic remodeling of the surviving myocardium after MI. Despite a growing body of knowledge regarding the changes in intracellular signaling that accompany post-MI remodeling [Feuerstein and Young, 2000; Woodcock et al., 2008; Dobaczewski and Frangogiannis, 2009; Hori and Nishida, 2009; Zamilpa and Lindsey, 2010], links between these empiric observations and the etiology of very complex and poorly understood chronic changes in cardiac myocyte (CM) biology that lead to macroscopic changes in ventricular structure and function over time in the post-MI heart remain elusive.

Correlative studies in human pathologic specimens have consistently indicated an upregulation in multiple signaling pathways during various stages of cardiac remodeling leading to heart failure [Ungerer et al., 1993; Abbate et al., 2002; Baba et al., 2003]. Although acute interventions in CM signaling are currently being contemplated as early responses to ischemic events, such molecular interventions would not be available to large numbers of patients who present much later in the course of their post-MI cardiomyopathy with chronic and evolving changes. Pathways that are believed to instigate a decline in myocardial function have been assumed to contribute to a pathologic process in chronic remodeling, whereas those believed to have an ameliorative effect, on the other hand, are assumed to represent a natural compensatory mechanism. It is imperative, however, to confirm these assumptions and to better understand the roles of specific signaling pathways in long-term ventricular remodeling in order to effectively design novel interventions that will be relevant to the treatment of millions of post-MI patients.

It has been postulated that either pharmacologic or genetic manipulation of various signaling pathways might succeed in

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reducing the progressive apoptotic loss of CMs and thereby lessen ventricular wall thinning and fibrosis during post-MI remodeling, the leading cause of human heart failure [Dorn, 2009]. We have shown recently, however, that a cell type-specific elucidation of molecular regulation in the diseased myocardium may be essential for successful application of this type of advanced targeted therapy, as non-specific induction of anti-apoptotic signaling in cardiac non-myocytes might actually enhance fibrosis rather than impede myocardial wall thinning [Yeh et al., 2010].

Multiple arms of the mitogen activated protein (MAP) kinase pathway have been shown to be upregulated during the development of post-MI remodeling. Data from our laboratory and others' have suggested that the balance between so-called stress activated kinases p38 and JNK and the putative anti-apoptotic kinases ERK1/2 may be disrupted during this pathologic progression, resulting in an increase in CM dysfunction and apoptosis [Zhang et al., 2003; Qin et al., 2005; Ren et al., 2005; Yeh et al., 2010]. Supraphysiologic upregulation of ERK1/2 signaling, achieved via CM-specific overexpression of a constitutively active form of the ERK activator kinase MEK1 (aMEK1), has been shown to prevent the acute functional loss of left ventricle through the reduction of the size of infarct 24 h after ischemia-reperfusion (I/R) [Bueno et al., 2000]. These observations regarding alterations of short-term bursts of pro- and anti-apoptotic signaling that contribute heavily to the survival of CMs after the acute insult of ischemia and reperfusion, however, have little bearing on long-term and complex changes in CM signaling during chronic post-MI remodeling. Given the greater complexity of pathologic stresses and the more prolonged time course of this pervasive pathologic entity, we undertook focused gain-of-function studies utilizing a transgenic mouse model of low-moderate CM-specific aMEK1 overexpression to establish the potential role of enhanced ERK1/2 signaling in ameliorating both the structural degeneration of the remote myocardium as well as progressive ventricular dysfunction during the evolution of post-MI cardiomyopathy.

METHODS

MOUSE CORONARY LIGATION

Eight to ten week old aMEK-1 transgenic (MEK1 Tg) mice (kindly provided by Dr. Jeffrey Molkentin) and their wild-type (WT) littermates were anesthetized with isoflurane prior to intubation with a 24 gauge catheter. Inhalation anesthesia was then instituted with 1.5% isoflurane using a rodent ventilator (Harvard) at 115 breath cycles/min. A left lateral thoracotomy incision was placed at the level of the fourth interspace and a 7.0 polypropylene suture was used to ligate the left anterior descending artery (LAD) at approximately 1/3 the distance from the base to the apex of the heart. The left chest was then closed and the animal was recovered after extubation in a light-warmed incubator. Animals were subsequently sacrificed at 4 weeks after infarction; at sacrifice, some hearts were fixed and sectioned for histologic analysis, while others were snap frozen for protein extraction. All procedures conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), and were approved by the Institutional Animal Care and Use Committee of the San Francisco Veterans Affairs Medical Center.

WESTERN BLOTTING AND GELATIN ZYMOGRAPHY

At the time of harvest of specimens for protein extraction, the left ventricle was dissected from the remainder of the heart. The infarct area was identified visually and dissected from the remainder of the myocardium. In addition, myocardial tissue from the free wall and septum clearly distant from the area of infarction was collected as remote myocardium. Any attempt to accurately isolate the irregular border zone from these small mouse hearts was felt to introduce a prohibitive sampling error. Specimens were snap frozen in liquid nitrogen. Myocardial tissues were homogenized in a lysis buffer containing 0.13 M KCl, 1 mM EDTA, 1 mM EGTA, 1 mM Na_3 (VO_4), 5 mM NaF, 20 mM HEPES, and Protease inhibitor cocktail tablet (Roche Diagnostics, Indianapolis, IN) and centrifuged at 35,000 rpm for 30 min. Supernatants were separated by gel electrophoresis, blotted onto a PVDF membrane (Invitrogen), and detected by chemiluminescence kit (Pierce) after incubation with primary antibody and corresponding horseradish peroxidase-labeled secondary antibody according to the manufacturer's instructions. To determine MMP activity, tissue lysates were applied to 10% Zymogram Gelatin Gel (Invitrogen) and electrophoresis was done according to the manufacturer's manual. After electrophoresis, the gels were washed twice in incubation buffer (50 mM Tris–HCl, 5 mM $CaCl_2$, 150 mM NaCl, and 0.05% NaN_3) for 20 min each at room temperature before incubation in fresh buffer overnight at 37°C. The gels were stained in 2% Coomassie Brilliant Blue G/25% methanol/10% acetic acid for 2 h and then destained for 1 h in 2% methanol/4% acetic acid. Gel images were captured by AlphaImager. Band densities were analyzed by Image J Software (Bethesda, MA).

ECHOCARDIOGRAPHY AND MILLAR CATHETERIZATION

Transthoracic echocardiography was performed in conscious mice using an Acuson Sequoia 512 machine and a 13-MHz probe. A two-dimensional short-axis view of the left ventricle was obtained at the level of the papillary muscles, that is, in myocardium remote to the infarction, and two-dimensional M-mode tracings were also recorded. LV fractional shortening was calculated as (LVDd–LVDs)/LVDd × 100, where LVDd = LV diastolic dimension and LVDs = LV systolic dimension [Yeh et al., 2010]. In vivo hemodynamic measurements were done in the anesthetized mice by inserting a 1.4 F Millar pressure–volume (PV) catheter into the LV camber via the right carotid artery [Pacher et al., 2008] and hemodynamic parameters were recorded and analyzed by Millar PV system MPVS-400 and PVAN program (Millar Instruments Inc.).

HISTOLOGY AND APOPTOSIS

Infarct area 24 h after LAD ligation was determined by triphenyltetrazolium chloride (TTC; Sigma-Aldrich) staining. Briefly, after perfusion with Evan's blue dye, each heart was removed and sliced horizontally into 2 mm slices. The slices were incubated in 1% TTC in phosphate buffered saline for 5 min at 37°C. The infarct ratio was determined by dividing the infarct area (white) by the total area-at-risk (white and red). Five-micron sections of pressure-fixed and paraffin-embedded hearts were stained with Gomori trichrome and

Sirius Red to assess LV infarct size and fibrosis in the remote myocardium. Myocyte size was evaluated via staining with Texas Red-conjugated wheat germ agglutinin (WGA, Invitrogen). All acquired images were analyzed and compared using the Image J Software (Bethesda, MA). Sections were also subjected to TUNEL staining (ApopTag Peroxidase In Situ Apoptosis Detection Kit, Chemicon, Temecula, CA), and apoptotic cells and total cells, identified via hematoxylin nuclear counterstaining, were counted in three sections/heart hematoxylin–eosin staining was used to distinguish the morphology of cells containing apoptotic nuclei on adjacent sections. The level of apoptosis is expressed as apoptotic cells as a percentage of total nuclei.

STATISTICS

Values are reported as mean ± SEM. Comparisons among groups were made using ANOVA, followed by Neuman–Keuls post-hoc testing. *P*-values lesser than 0.05 were considered statistically significant, with Bonferroni correction where appropriate.

RESULTS

TRANSGENIC MODEL OF LOW–MODERATE CM-SPECIFIC aMEK1 EXPRESSION

Although the cardiac-specific upregulation of MEK-ERK signaling has been linked to stable, physiologic hypertrophy of the myocardium, the level of constitutive aMEK1 expression has been shown to impact the resulting phenotype. Whereas a high level of expression is associated both with an increase in myocardial mass and a demonstrable improvement in functional parameters such as fractional shortening, lower expression levels have been found to have a much more moderate increase in myocardial wall thickness and overall muscle mass [Bueno et al., 2000]. Given our interest in the impact of MEK-ERK signaling on chronic, post-MI remodeling, we hoped to minimize the confounding effects of pre-MI hypertrophy and of MEK-ERK signaling during the evolution of acute infarction that are inevitably associated with a transgenic model of constitutive CM-specific expression. We therefore worked with a model of low to moderate constitutive aMEK1 expression that minimized the difference in pre-MI cardiac structure and function between our experimental Tg animals and their littermate, WT controls.

Low to moderate CM-specific aMEK1 expression resulted in a 21% increase in HW/BW ratio, compared to the 11% and 37% increases observed previously in low and intermediate CM aMEK1 overexpression, respectively [Bueno et al., 2000]. Constitutively high aMEK1 expression leads to a 23% and 45% reduction in baseline LV end diastolic and end systolic dimensions, respectively, while our MEK1 Tg mice did not display any reduction in these baseline (i.e., pre-infarct) parameters of LV structure. Furthermore, whereas high levels of aMEK1 expression lead to a measurable 33% increase in both fractional shortening and LV dP/dt_{max} [Bueno et al., 2000], our aMEK1 transgenics did not display any difference in baseline cardiac contractile function compared to WT littermate controls (Table I).

REGULATION OF MPAK SIGNALING IN THE POST-MI REMOTE MYOCARDIUM

Persistent expression of a constitutively active form of MEK1 in CM was associated not only with an increase in ERK 1/2 phosphorylation (i.e., activation), but also with an interesting increase in total ERK 1/2 protein expression. Although a similar increase in ERK protein expression was observed previously in the MEK1 Tg [Bueno et al., 2000], the mechanism of MEK1 regulation of ERK 1/2 expression has yet to be elucidated. Upregulation of ERK1/2 protein expression after MI, however, was substantially higher in the myocardium of non-transgenic mice (Fig. 1) compared to aMEK1 transgenic littermates. In contrast, the amount of phosphorylated ERK 1/2 (P-ERK) was significantly higher in MEK1 Tg compared to littermate WT controls in stressed cardiac cells after MI (Fig. 1). Expression of MAP kinase phosphatases (MKPs) 1 and 3 did not differ between the myocardial tissues of MEK1 Tg and WT controls after MI, suggesting that this difference in ERK phosphorylation was not mediated by an alteration in MKP expression. Levels of stress activated kinase p38 and JNK expression and phosphorylation were not significantly different in MEK1 Tg compared to WT controls.

TABLE I. Functional Parameters of the Left Ventricles 4 Weeks After LAD Ligation

	SHAM		MI	
	MEK1 Tg (n = 13)	WT (n = 14)	MEK1 Tg (n = 45)	WT (n = 45)
FS (%)	52 ± 1	52 ± 1	49 ± 1†	43 ± 1†
LVEDV (μl)	39 ± 4	36 ± 3$^{\neq}$	42 ± 3†	54 ± 4$^{\dagger\neq}$
LVESV (μl)	9 ± 1	8 ± 1$^{\neq}$	11 ± 1†	17 ± 2$^{\dagger\neq}$
SW (mmHg × μl)	NA	NA	732 ± 141*	371 ± 91*
dPdt max (mmHg/s)	NA	NA	5341 ± 628	4257 ± 438
Ees (mmHg/μl)	NA	NA	6.14 ± 0.57*	3.92 ± 0.6*
PRSW (mmHg)	NA	NA	603 ± 98*	335 ± 40*
βMHC/αMHC</bold	NA	NA	1.20 ± 0.08*	1.37 ± 0.09*
ANP/18S	NA	NA	1.29 ± 0.13*	1.54 ± 0.11*
HW/BW (%)	0.58 ± 0.02†	0.48 ± 0.01†	0.59 ± 0.01†	0.51 ± 0.01†

$^{*}P < 0.05$; $^{\dagger\neq}P < 0.01$; *†Tg versus WT; $^{\neq}$sham versus MI; for in vivo hemodynamic measurements, n = 9 in MEK1 Tg and n = 7 in WT; for real-time PCR analysis of the expression of MHCs and ANP, n = 13 in MEK1 Tg and n = 4 in WT. FS, fractional shortening; LVEDV, left ventricle end-diastolic volume; LVESV, left ventricle end-systolic volume; SW, stroke work; Ees, end-systolic elastance; PRSW, preload recruitable stroke work.

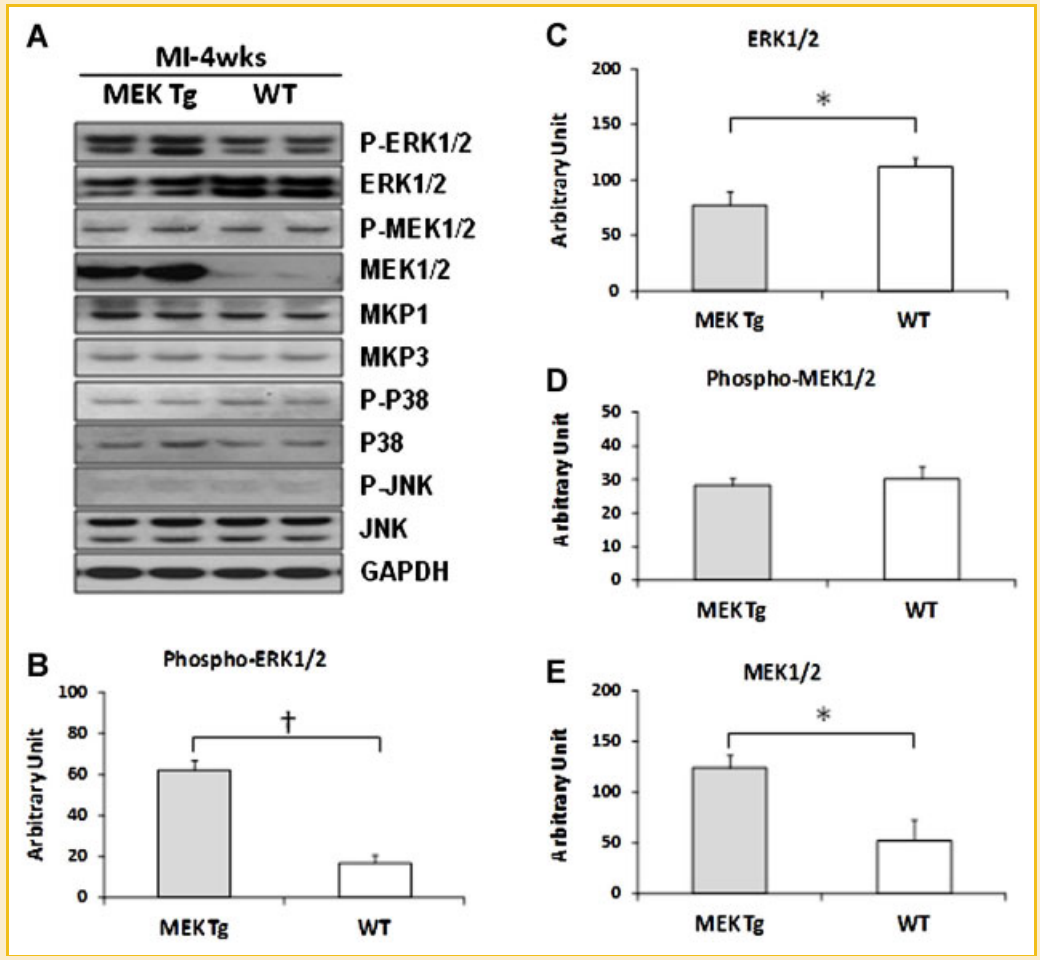

Fig. 1. Expression of MAPK related proteins in the remote myocardium 4 weeks after MI. A: Representative Western blots of protein expression and phosphorylation of MAPK-related proteins. B–E: Quantification of phosphorylated ERK1/2 (P-ERK1/2), total ERK1/2, phosphorylated MEK1/2 (P-MEK1/2), and total MEK1/2 expression levels ($n = 14$, MEK1 Tg; $n = 7$, WT; $^{*}P < 0.05$).

REDUCTION IN MYOCARDIAL APOPTOSIS, PERSISTENCE OF CM HYPERTROPHY AFTER MI

It has previously been documented that post-MI remodeling of the remote myocardium is associated with a significant increase in myocardial apoptosis [Qin et al., 2005; Yeh et al., 2010]. The large increase in P-ERK in MEK1 Tg hearts compared to littermate WT controls was associated with a statistically significant 59% reduction in myocardial apoptosis (apoptotic rate of $0.66 \pm 0.11\%$ in MEK1 Tg vs. $1.60 \pm 0.34\%$ in littermate WT controls, $P < 0.05$), as measured by TUNEL staining (Fig. 2A). Furthermore, it has been shown that constitutive MEK1-ERK1/2 signaling in the uninfarcted hearts of these transgenic mice results in a stable hypertrophy of CMs that does not degenerate into a dilated cardiomyopathy. Even after MI, surviving CMs in the remote myocardium of MEK1 Tg mice remained significantly larger than the CM of littermate WT controls (Fig. 2B).

REDUCTION IN MYOCARDIAL INFARCT AREA AND FIBROSIS

The loss of CMs to a slow but relentless increase in apoptosis is believed to contribute to chronic extension of infarction in the LV wall, and to weakening and fibrosis of the post-MI remote myocardium. Reperfusion injury involves a significant degree of CM apoptosis that contributes to acute infarct size. Even though overexpression of anti-apoptotic aMEK1 has been shown to protect the myocardium from acute reperfusion injury and reduce acute infarct size in the area-at-risk, we found that aMEK1 overexpression did not affect the initial infarct size 24 h after permanent ligation of the LAD in which necrosis, rather than apoptosis, predominantes and in which there is little reperfusion injury (Fig. 2C, acute infarction of $57.5 \pm 4.7\%$ and $54.0 \pm 4.6\%$ of the area-at-risk in MEK1 Tg and WT controls, respectively, $P = 0.96$). However, at 4 weeks post-MI the percentage of infarct in the LV wall was significantly lower in MEK1 Tg mice than in their littermate WT controls (Fig. 2D). Fibrosis was also found to be substantially reduced in the remote myocardium of post-MI MEK1 Tg mice compared to their littermate controls (Fig. 2E).

CHANGES IN MATRIX-MODULATING PROTEINS

The role of matrix metalloproteinases (MMPs) and tissue inhibitors of metalloproteinases (TIMPs) in pathologic remodeling remains

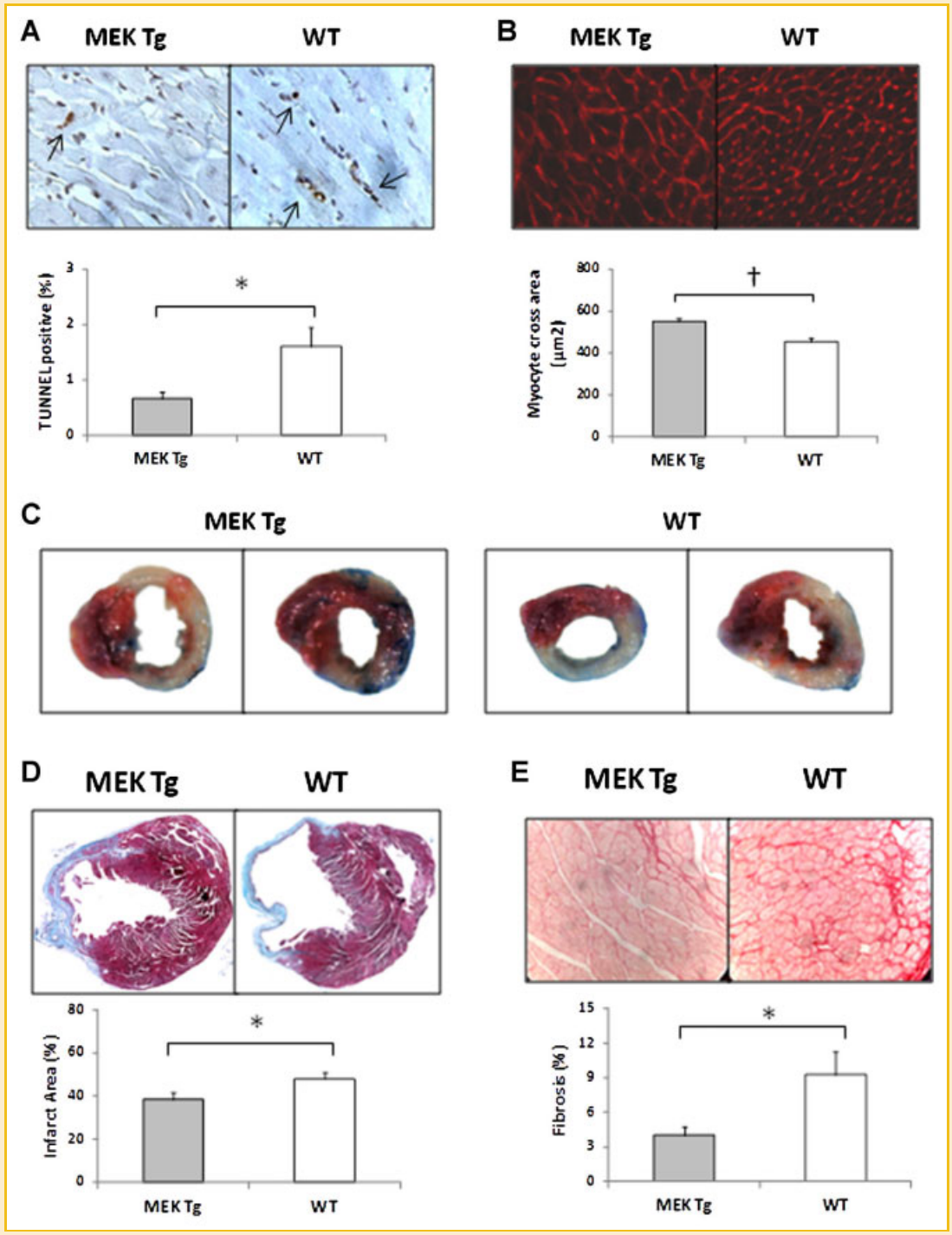

Fig. 2. Histological changes in MEK1 Tg and WT remote myocardium 4 weeks after MI. A: Apoptosis by TUNEL staining (400×). B: Myocyte surface revealed by WGA staining (100×), C: Infarct size in the risk area measured by TTC staining (10×). D: Percentage of LV infarction measured via trichrome staining (20×). E: Fibrosis as measured by collagen staining (100×). n = 5–10, $^{*}P < 0.05$, $^{\dagger}P < 0.01$, NS, no significant difference.

somewhat controversial, but specific MMPs and TIMPs have been suggested by some to moderate the accumulation of collagen during myocardial fibrosis [Baker et al., 1998; Hayashidani et al., 2003; Matsusaka et al., 2005; Mias et al., 2009; Givvimani et al., 2010]. Although the protein levels of MMP2, known to play an important role in the myocardium and associated in some studies with protection from fibrotic remodeling, were similar in infarcted hearts from both MEK1 Tg and WT hearts, the activity of this enzyme was significantly higher in the post-MI remote myocardium of the transgenic animals (Fig. 3). Similarly higher MMP2 activity was also observed in non-infarcted MEK1 Tg hearts than in non-transgenic control hearts, suggesting a possible role for MMP2 in the resistance

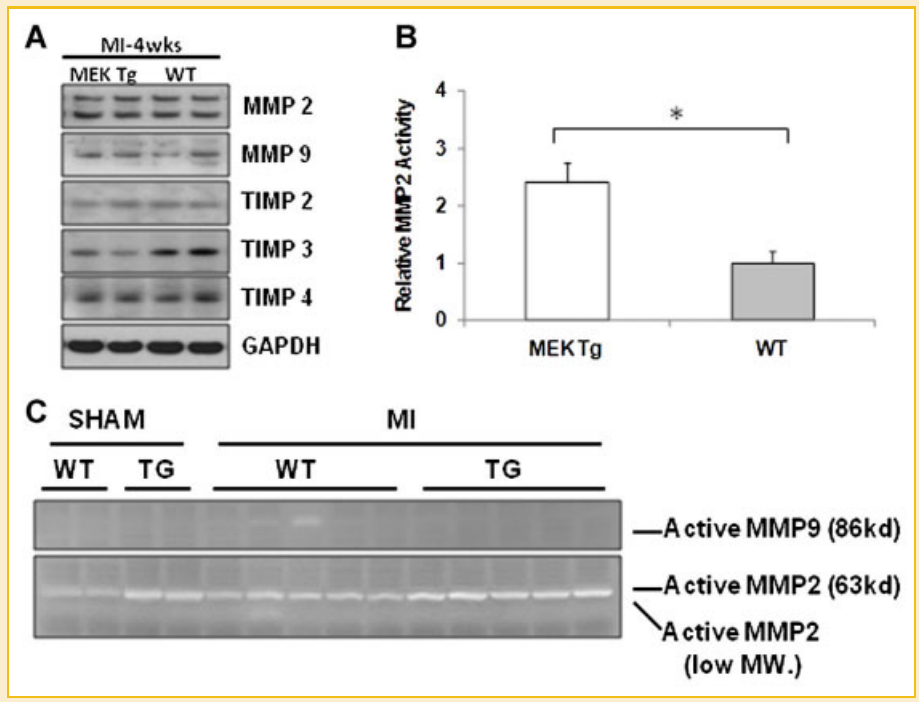

Fig. 3. Expression of MMP-related proteins in the remote myocardium 4 weeks after MI. A: Representative Western blots of protein expression of MMPs and TIMPs. B: Quantification of MMP2 activity in the remote myocardium after MI ($n = 5$; $^{*}P < 0.05$). C: Representative zymography of MMP2 and MMP9 activity in the remote myocardium of MEK1 Tg and WT mice with or without MI.

that has been demonstrated in these hearts to deterioration from stable to pathologic hypertrophy. MMP9 and TIMP3 have been associated with pathologic remodeling in some studies [Baker et al., 1998; Givvimani et al., 2010]; interestingly, both higher MMP9 activity (in some animals) and significantly higher expression of TIMP3 (in all animals tested) were observed in the remote myocardium of non-transgenic infarcted hearts, but not in the infarcted hearts of MEK1 Tg mice (Fig. 3).

PRESERVATION OF LEFT VENTRICULAR (LV) MACROSCOPIC STRUCTURE

Echocardiography of MEK1 Tg animals after MI reflected a preservation of global LV structure, with no significant increase in either diastolic or systolic LV dimensions compared to non-infarcted controls (Table I). In contrast, the LV chambers of post-MI WT hearts were dilated, with 50% ($P < 0.01$) and 113% ($P < 0.01$) increases in LV diastolic and systolic dimensions, respectively (Table I).

PRESERVATION OF REGIONAL AND GLOBAL LV FUNCTION

The preservation of microscopic and macroscopic structure in the remote myocardium of post-MI MEK1 Tg mice also coincided with a significant preservation of LV function compared to WT controls. A 17.3% decline in fractional shortening (regional function) was observed at a point remote from the LV infarct in the myocardium of WT controls. In contrast, this decline was only 6% in hearts with CM-specific aMEK1 overexpression during the post-MI period (Table I). This functional benefit from MEK1-ERK1/2 gain-of-function was confirmed with invasive measurements of stroke work (SW), end-systolic elastance (Ees), and preload recruitable stroke work (PRSW), confirming a significant improvement in ventricular contractility after MI compared to non-transgenic littermates (Table I).

DOWNSTREAM MOLECULAR PATHWAYS

The phosphorylation (i.e., activation) of various known downstream mediators of MEK1-ERK1/2 signaling was assessed in an attempt to better understand the therapeutic mechanism of aMEK1 over-expression in reducing post-MI remodeling of the remote myocardium. A significant increase was observed in the phosphorylation of pro-survival transcription factor STAT3 (Fig. 4). The increase in absolute levels of P-ERK1/2 in MEK1 Tg hearts was also associated with an increase in protein levels of anti-apoptotic Bcl-2 and in phosphorylation (i.e., inactivation) of pro-apoptotic BAD, while expression of anti-apoptotic Bcl-xL and pro-apoptotic protein BAX remained the same (Fig. 5).

DISCUSSION

Although previous studies have begun to address the causal and potentially therapeutic roles of changes in MEK-ERK signaling in the setting of acute ischemia and reperfusion, the objective of these studies was an elucidation of the impact of myocardial MEK-ERK activation on chronic post-MI remodeling, possibly the largest

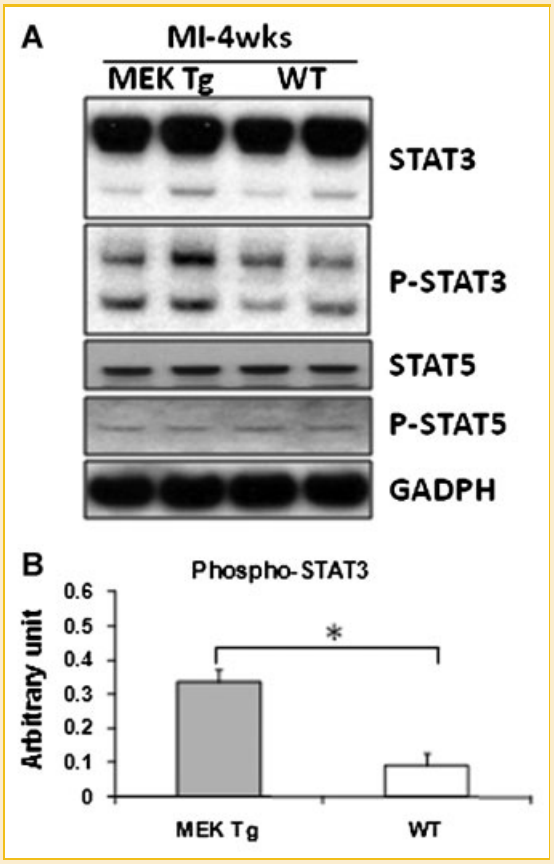

Fig. 4. Expression of STATs in the remote myocardium 4 weeks after MI. A: Representative Western blots of protein expression and/or phosphorylation (P-) of STAT3 and STAT5. B: Quantification of phospho-STAT3 (P-STAT3) expression and phosphorylation level in the post-MI remote myocardium, $n \geq 6$, $^{*}P < 0.05$.

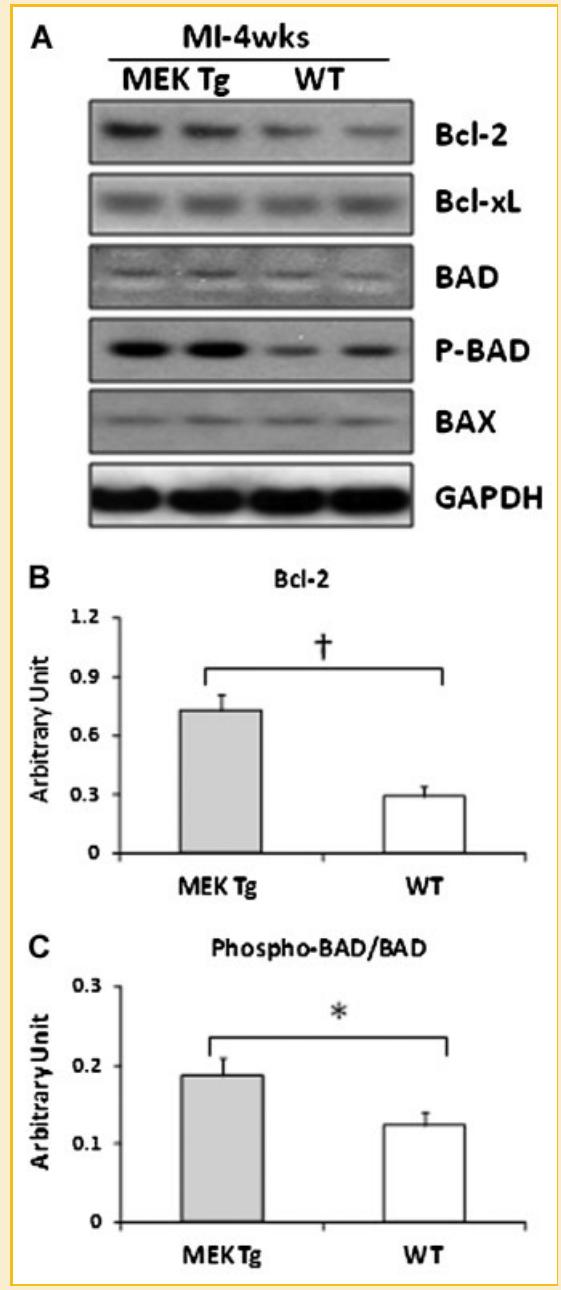

Fig. 5. Regulation of apoptosis-related genes in the 4 weeks post-MI remote myocardium. A: Representative Western blots of protein expression and/or phosphorylation (P-) of anti-apoptotic and pro-apoptotic factors. B–C: Quantification of Bcl-2 and phosphorylated BAD/BAD level in the post-MI remote myocardium, $n \geq 6$, $^{*}P < 0.05$, $^{\dagger}P < 0.01$.

single cause of congestive heart failure in the United States. While these previous studies have demonstrated a role for changes in MEK-ERK signaling in ameliorating the apoptosis and extent of infarction related to very rapid changes instigated by acute stresses in CM, they do not speak to the possible role of long-term alteration in the balance of MAPK pathways in the much more complex and insidious process of chronic ventricular fibrosis and remodeling. We worked with a transgenic mouse model in which low to moderate levels of constitutive aMEK1 expression from birth led only to a moderate increase in baseline HW/BW ratio and individual CM surface size, but no other demonstrable differences in other parameters of myocardial structure or function prior to a standardized permanent coronary ligation. The prior influence of low–moderate over-expression of MEK-ERK signaling in this model before MI may therefore have had only a minimal confounding effect on the chronic remodeling observed after MI, allowing at least tentative conclusions to be drawn from the effect of post-MI MEK-ERK overexpression on this important pathologic process.

In this study, a transgenic model of stable, long-term CM-specific upregulation of MEK1-ERK1/2 signaling did, in fact, suggest that modulation of the balance among MAP kinase pathways can play a role in the context of chronic LV remodeling. Overexpression of aMEK1 resulted in a reduction in myocardial apoptosis, documented via TUNEL staining, and was associated with increased levels of anti-apoptotic Bcl-2 protein and of phosphorylation (inactivation) of pro-apoptotic BAD. The chronic protection against CM apoptosis afforded by long-term P-ERK1/2 upregulation may also have been mediated, at least in part, by an observed increase in phosphorylation (activation) of the cardioprotective transcription factor STAT3 [Obana et al., 2010].

Chronic inhibition of apoptosis in the surviving post-MI myocardium was correspondingly associated both with a substantially lower degree of infarct extension and with an inhibition of post-MI fibrosis in the remote myocardium. Although the expression and activity of MMP2 has been linked to the rupture of infarcted myocardium in acute MI models, the role of MMP2 in the uninfarcted remote myocardium during chronic remodeling remains to be fully elucidated [Hayashidani et al., 2003]. In fact, MMP2, the activity of which was increased in the remote post-MI myocardium of MEK1 Tg mice, has been shown to suppress inflammation and preserve cardiac function in a model of TNF-induced cardiomyopathy, and may also promote adaptive remodeling via the removal of excess collagen from the region remote to a chronic infarct [Matsusaka et al., 2005; Mias et al., 2009]. In fact, nonspecific inhibition of MMP activity has been found to enhance pathological hypertrophy in the mouse aortic constriction model [Vinet et al., 2008]. Another recent study suggested that MMP2 may promote angiogenesis during adaptive hypertrophy [Givvimani et al., 2010]. On the other hand, increased expression of TIMP3 has been shown to induce apoptosis, and both TIMP3 and MMP9 have been found to contribute to decompensatory hypertrophy [Baker et al., 1998; Givvimani et al., 2010]. Both of these potentially pathologic modulators of myocardial matrix were found to be elevated in specimens from WT but not MEK1 Tg mice after MI. It has been reported that aMEK1 can induce both MMP2 and MMP9 protein translation and increase their activities [Lemieux et al., 2009], but only an increase in MMP2 activity was observed in MEK1 Tg after MI. This observation of increased MMP2 and a possible change in the balance among different MMPs and TIMPs in MEK1 Tg mice may contribute to a favorable environment for maintaining compensatory hypertrophy rather than pathologic hypertrophy in the post-MI myocardium.

Extensive in vitro studies of cardiac myocyte biology have documented a well established role for MEK1 in instigating a downstream cardioprotective cascade involving ERK1/2 phopshorylation, both through pharmacologic and genetic MEK1 inhibition [Zhu et al., 1999; Yue et al., 2000; Huang et al., 2007], and through MEK1 gain of function [Bueno et al., 2000]. In addition, augmentation of ER1/2 phosphorylation through down regulation of MKP3 has also been associated with enhanced cell survival and adaptive cardiac hypertrophy [Maillet et al., 2008]. These and other studies, however, have demonstrated that changes in MEK1-ERK1/2 signaling develop rapidly (on the order of minutes) in the context of acute hypoxia [Seko et al., 1996]; rapid changes in ERK1/2 phosphorylation have similarly been observed in ex vivo and in vivo studies of I/R injury [Knight and Buxton, 1996; Ballard-Croft et al., 2006]. Cardiac upregulation of P-ERK1/2 in MEK1 Tg mice has also been found to reduce infarct size after I/R [Bueno et al., 2000]. Whereas it is understandable that rapid changes in MEK1-ERK1/2 signaling influence the balance of pro-apoptotic and survival signaling within cardiac cells in this acute setting of CM death immediately after I/R, very little is known about the functional significance of chronic changes in this signaling pathway as it relates to the insidious loss of CM during longer-term post-MI remodeling of the remote, uninfarcted myocardium. It is this chronic remodeling of the post-MI heart, however, that instigates the transition from compensated function after MI to cardiomyopathy in the majority of post-MI heart failure patients. The results of this study are among the first to directly suggest via gain-of-function that a causal relationship exists between P-ERK1/2 levels and the progression of myocardial apoptosis, fibrosis, and dysfunction during this chronic remodeling process. Given the enormous complexity of the stresses and of the biological responses within the myocardium during this chronic process, this kind of cell-type specific molecular elucidation is essential for confirming hypotheses regarding the contributory roles of various signaling moieties both to pathogenesis and to potential therapeutic molecular intervention.

Significant limitations remain, however, regarding the conclusions that can be drawn from these first-generation gain-of-function experiments. Although our model of low–moderate aMEK1 expression did minimize the impact of constitutive MEK1-ERK1/2 upregulation on pre-MI structure and function, the potentially confounding variable of modest, pre-existing cardiac hypertrophy in MEK1 Tg hearts at the time of acute infarction in this model could not be entirely eliminated. It cannot therefore be determined from this model what role, if any, was played by this modest pre-existing difference in myocardial structure on the outcome of post-MI remodeling. Measurement of acute infarct size in this model of permanent coronary ligation, in which apoptosis likely plays a smaller role relative to necrosis than in models of I/R, did not reveal a strong influence of anti-apoptotic ERK signaling on immediate infarct size. The larger infarcts seen at 4 weeks therefore likely reflect the contribution of this anti-apoptotic pathway in limiting more chronic infarct expansion.

Despite their limitations, the current studies have established, for the first time, an important verification of the contributory role that chronic changes in MEK1-ERK1/2 signaling can have on the complex, slow processes of CM loss and of regional/global changes in ventricular structure and function during pathologic cardiac remodeling. They also provide a necessary proof-of-concept with regard to the efficacy of molecular intervention in post-MI cardiomyopathy. Whereas previous studies have begun to explore therapeutic roles for manipulation of MAP kinase and other signaling pathways in the setting of acute ischemia and I/R [Bueno et al., 2000; Ren et al., 2005; Engel et al., 2006; Li et al., 2006], a greater impact on human heart failure may eventually be achievable through novel strategies that address the large pool of patients who live with and eventually succumb to chronic, post-MI remodeling of the remote myocardium.

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