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Basic Research

Macrophage-Specific IκB Kinase α Contributes to Ventricular Remodelling and Dysfunction After Myocardial Infarction

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ABSTRACT

Background: The IκB kinase (IKK) complex has been found to have critical functions in cancer and the immune system. In particular, IKKα, which is a member of the IKK complex, has been shown to influence the inflammatory response and malignant diseases. However, the role of IKKα in macrophages after myocardial infarction (MI) remains largely unknown.

Methods: Sham or MI operations were performed on macrophage-specific IKKα knockout (mIKKα$^{-/-}$) mice and IKKα$^{flox/flox}$ littermates. We ligated the left anterior descending coronary artery of the MI group and observed the results at 3, 7, and 30 days after MI.

Results: We discovered more severe cardiac dysfunction with reduced angiogenesis, fibrosis, and collagen deposition in mIKKα$^{-/-}$ than in IKKα$^{flox/flox}$. In addition, we also observed that macrophages in mIKKα$^{-/-}$ were easier to polarize to the M1 phenotype and expressed more proinflammatory factors than IKKα$^{flox/flox}$. Mechanistically, IKKα deficiency in macrophages inhibited the alternative nuclear factor-κB/RelB pathway and enhanced the MEK1/2/ERK1/2 pathway.

RÉSUMÉ

Introduction : Il a été montré que le complexe IκB kinase (IKK) assure des fonctions essentielles dans le cancer et le système immunitaire. Plus précisément, l'IKKα, qui fait partie du complexe IKK, s'est avérée avoir une influence sur la réponse inflammatoire et les affections malignes. Le rôle de l'IKKα dans les macrophages après un infarctus du myocarde (IM) reste néanmoins en grande partie inconnu.

Méthodologie : Des chirurgies fictives ou pour un IM ont été pratiquées sur des souris dont le complexe IKKα spécifique des macrophages avait été désactivé (mIKKα$^{-/-}$) et sur des congénères IKKα$^{flox/flox}$ de la même portée. Nous avons ligaturé l'artère coronaire descendante antérieure gauche dans le groupe IM et observé les résultats 3, 7 et 30 jours après l'IM.

Résultats : Nous avons noté une dysfonction cardiaque plus grave avec réduction de l'angiogenèse, fibrose et dépôts de collagène chez les sujets mIKKα$^{-/-}$, comparativement aux sujets IKKα$^{flox/flox}$. Nous avons également noté qu'il était plus facile de polariser les macrophages des souris mIKKα$^{-/-}$ vers le phénotype M1, et que celles-ci

Myocardial infarction (MI) is one of the leading causes of morbidity and mortality globally.[1] There is some evidence to indicate that inflammatory reactions play an essential role during the healing process and scar formation after MI.[2] The acute inflammatory response after MI is predominantly composed of neutrophil and macrophage infiltration.[3,4] Monocytes/macrophages are the primary responders for inflammation and very important for cardiac healing after MI at multiple levels.[5] During MI, macrophages infiltrate the necrotic myocardium, which results in a robust inflammatory response that is critical for scar formation and ventricular remodelling.[6] Furthermore, the molecular mechanisms that regulate gene expression during MI are still not completely understood.

Macrophages have been reported as a regulator of wound healing events, including cell debris clearance, infarct scar formation, and angiogenesis.[7] Macrophages are a kind of plastic immune cell, and their polarization is mainly classified into 2 types, including the classical type (M1) and alternative type (M2), which are distinguished by different cell markers. During the early inflammatory stage of MI, M1 macrophages display a strong inflammatory phenotype by infiltrating the necrosis area and secreting large amounts of proinflammatory cytokines, such as tumor necrosis factor-α (TNF-α),

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Conclusions: Overall, our data identified IKKα in the heart as a novel mediator that protected the heart from a severe inflammatory response and attenuated ventricular remodelling after MI by negatively regulating macrophage polarization to the M1 phenotype. Therefore, IKKα may serve as a potential therapeutic target for treatment after MI.

exprimaient davantage de facteurs pro-inflammatoires, comparativement aux souris IKKα$^{flox/flox}$. D'un point de vue mécanistique, la carence en IKKα des macrophages a inhibé la voie de signalisation alternative κB/RelB du facteur nucléaire et accentué la voie MEK1/2/ERK1/2.

Conclusions : D'une manière générale, nos données indiquent que l'IKKα présente dans le cœur constitue un médiateur novateur, qui protège le cœur contre la réponse inflammatoire marquée et diminue le remodelage ventriculaire après un IM, en régulant à la baisse la polarisation des macrophages vers le phénotype M1. L'IKKα pourrait donc constituer une cible potentielle du traitement après un IM.

interleukin-1β (IL-1β), and IL-6, which have the phagocytosis of cellular debris. However, if the proinflammatory response becomes excessive or uncontrolled, it will lead to a severe inflammatory reaction and inhibit the healing process. M2 macrophages are marked by molecules, such as transforming growth factor-β, IL-10, arginase 1 (Arg1), and Mrc1,[8] that have anti-inflammatory functions and promote tissue regeneration.[9,10] In contrast to the M1 phenotype, M2-polarized macrophages mitigate cardiac functional deterioration and improve infarct repair after MI.[11]

The nuclear factor-κB (NF-κB) pathway is a main regulator of innate and acquired immunity. The IκB kinase (IKK) complex is a key regulator of NF-κB that is composed of 2 catalytic subunits, IKKα and IKKβ, and the regulatory subunit IKKγ/NF-κB essential modulator. There are 2 NF-κB pathways: the classical pathway and the alternative pathway. The classical pathway is mainly activated by phosphorylation of IKKβ, which leads to the nuclear translocation of the NF-κB factor (especially the p65/p50 complex). However, the alternative NF-κB pathway is mediated independently of IKKβ or NF-κB essential modulator but requires the activation of IKKα. Activated IKKα phosphorylates p100 or RelB, which translocate to the nucleus and activate transcription.[12] Further study of the mechanism showed that IKKβ can inhibit activation of the M1 macrophage phenotype,[13] whereas macrophages tend to secrete proinflammatory factors when they lack IKKα.[14-16] Inflammation plays an important role in heart disease. IKK and NF-κB have been confirmed as regulators of inflammatory reaction and cardiac disorders. Recently, the functions of macrophage influencing cardiac repair and ventricular remodelling after MI have also received more focus. For example, macrophages play an important role in cardioprotection, fibrosis, collagen deposition, and degradation of the extracellular matrix, because of their plasticity.[17,18] However, the possible roles of IKKα in MI have not received as much attention from researchers.

In this study, we focus on the IKKα that regulates macrophage polarization after MI. We found that macrophage-specific IKKα knockout (mIKKα$^{-/-}$) had a more severe myocardial inflammatory reaction and more attenuated ventricular remodelling, cardiac function recovery, fibrosis, collagen deposition, and angiogenesis than IKKα$^{flox/flox}$. The mIKKα$^{-/-}$ expressed more proinflammatory factors and fewer anti-inflammatory factors than IKKα$^{flox/flox}$, which demonstrated that the macrophages in mIKKα$^{-/-}$ were easier to polarize to the M1 phenotype than macrophages in IKKα$^{flox/flox}$. The molecular signalling pathway analysis demonstrated that the expression of phos-MEK1/2, phos-ERK1/2, and MEK1/2/ERK1/2 increased at both 3 and 7 days after macrophage deficiency of IKKα after MI, while phos-RelB decreased. Taken together, our findings implicate IKKα as a potential therapeutic target for ischemic heart disease.

Methods

For detailed materials and methods, see the Supplementary Material.

Results

IKKα was overexpressed in macrophages in the infarct zone after MI

To investigate the potential role of IKKα in processes after MI, we first examined whether IKKα expression levels were altered in diseased hearts. At the beginning, we ligated the left anterior descending of C57B6 mice in the MI group for 3, 7, and 30 days. We found the expression of IKKα dramatically increased after permanent ligation of the left coronary artery compared with the sham hearts, especially at 7 days. However, there was no compensatory alteration of IKKβ and IKKγ during the whole process, either at baseline or after MI (Fig. 1A). Moreover, coimmunofluorescence analysis confirmed phos-IKKα and CD68 showed colocalization of phos-IKKα in the nuclei of macrophages during the first 7 days after MI (Fig. 1B). Collectively, the alterations of IKKα levels in macrophages in murine infarcted hearts indicate that IKKα may be involved in postinfarction ventricular remodelling.

IKKα deficiency in macrophages led to increased mortality, aggravated infarct size expansion, and cardiac functional deterioration after MI

After the ligation of left anterior descending in both operation groups, the mortality of mIKKα$^{-/-}$ was higher than IKKα$^{flox/flox}$, especially from 5 to 10 days (Fig. 2A). The most common cause of death was heart rupture at the infarct area. The survival rate of mIKKα$^{-/-}$ was only approximately 25%, which was much lower than IKKα$^{flox/flox}$ (50%). Kaplan-Meier curves revealed that the high mortality occurred during the first week after MI, which indicated there was a selective detrimental effect of IKKα deficiency in macrophages at this stage. The images of positron emission

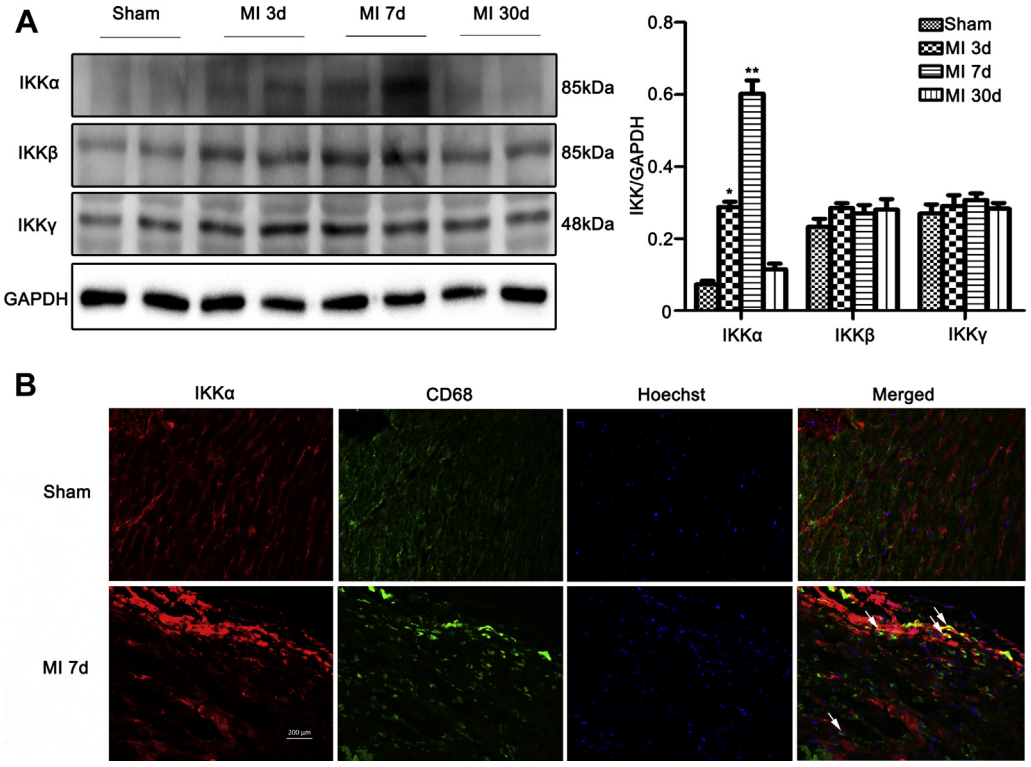

Figure 1. IκB kinase α (IKKα), but not IKKβ or IKKγ, expression is high in heart tissue after myocardial infarction (MI), especially at 7 days. (**A**) Representative western blot and quantitative results showing expression of IKKα, IKKβ, and IKKγ in heart tissue at 3, 7, and 30 days after MI (n = 4 mice per experimental group, $*P < 0.05$ vs IKKα Sham, IKKα MI 7d, and IKKα MI 30d; $**P < 0.01$ vs IKKα Sham, IKKα MI 3d, and IKKα MI 30d). (**B**) Coimmunofluorescent staining presenting the expression of phos-IKKα (**red**) and CD68 (**green**) in the myocardium at 7 days after MI (**arrows** indicate cells expressing both IKKα and CD68; n = 3 independent experiments, ×400).

tomography-computed tomographyalso demonstrated a more extensive infarct zone in mIKKα$^{-/-}$ than in IKKα$^{flox/flox}$ control subjects at 30 days after MI (Fig. 2B). As expected, the echocardiographic analysis of cardiac function showed a significant decrease in the ejection fraction, fractional shortening, and interventricular septum diastole as well as a decrease of left ventricular (LV) internal dimension diastole in mIKKα$^{-/-}$ at 30 days after MI, which demonstrated the acceleration of cardiac dilatation and deterioration of LV function in mIKKα$^{-/-}$ compared with IKKα$^{flox/flox}$ (Fig. 2, C and D). Although quantitative testing of MI markers in serum, such as troponin T, creatine kinase-MB (CK-MB), and lactate dehydrogenase (LDH), revealed no differences between the 2 groups, markers of fibrosis revealed an obvious decrease in hyaluronidase, type III procollagen peptide, type IV collagen, and laminin in the serum of mIKKα$^{-/-}$ compared with IKKα$^{flox/flox}$ at 30 days after MI (Supplemental Fig. S1). In addition, hematoxylin-eosin (HE) staining revealed that mIKKα$^{-/-}$ had a larger infarct size (80%) with a much thinner wall thickness at the infarct area than IKKα$^{flox/flox}$ (infarct size: 50%) at 30 days after MI (Fig. 2E). Although the infarct zone was similar between mIKKα$^{-/-}$ and IKKα$^{flox/flox}$ at 7 days, the fibrosis level and collagen deposition in IKKα$^{flox/flox}$ mice were much higher than mIKKα$^{-/-}$. Thus, these data demonstrate that IKKα in macrophages has a protective effect on survival rates, infarct size, and cardiac function after MI.

IKKα deficiency in macrophages attenuates scar formation and collagen deposition after MI

We further investigated the effects of IKKα on scar formation and collagen deposition. Masson trichrome staining revealed that mIKKα$^{-/-}$ had substantially increased fractions of the collagen area/LV area at 30 days after MI (Fig. 3A). Although the fraction was high, the LV wall thickness and density of collagen were extremely low in mIKKα$^{-/-}$. As expected, the expression of alpha-smooth muscle actin (α-SMA), collagen I, fibronectin, and vimentin were dramatically limited in mIKKα$^{-/-}$, but they were markedly exaggerated in IKKα$^{flox/flox}$ at 7 and 30 days (Fig. 3, B and C). Those phenotypes indicate that mIKKα$^{-/-}$ have more attenuated scar formation and angiogenesis with less fibrosis and collagen deposition than IKKα$^{flox/flox}$, and these data indicate that IKKα expression in macrophages protects against postinfarction LV remodelling.

IKKα deficiency in macrophages facilitates proinflammatory factors and regulates macrophage polarization to the M1 phenotype at 7 days after MI

Given that macrophages can polarize to different phenotypes with adverse functions by secreting proinflammatory or anti-inflammatory factors, we tested some inflammatory factors and representative markers of macrophage M1 and M2 phenotypes by q-PCR. The amounts of proinflammatory

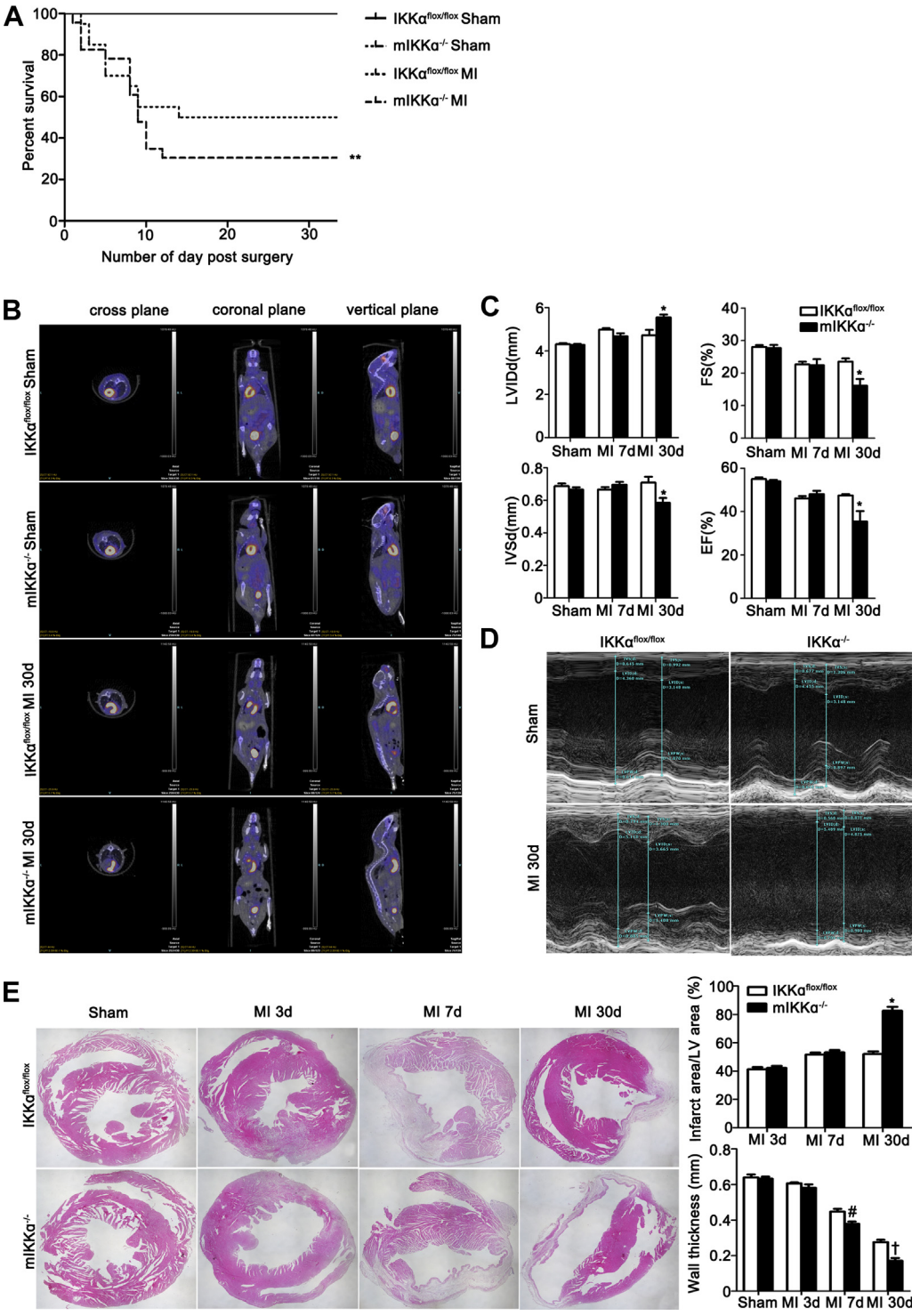

Figure 2. Macrophage IκB kinase α (IKKα) deficiency decreases survival rate, increases infarction area, and aggravates cardiac dysfunction at 30 days after myocardial infarction (MI). **(A)** Survival rate of macrophage-specific IKKα knockout (mIKKα$^{-/-}$) and IKKα$^{flox/flox}$ mice in the sham or MI group (n = 30 mice per experimental group, $^{**}P < 0.01$ vs IKKα$^{flox/flox}$ and mIKKα$^{-/-}$ Sham). (**B**) Images of positron emission tomography-computed tomography of mIKKα$^{-/-}$ and IKKα$^{flox/flox}$ show the area of the activated myocardium (ie, the light area in the chest) at 30 days after MI from different planes (n = 4 mice per experimental group). (**C, D**) Representative images and parameters of echocardiography (LVIDd, IVSd, EF, FS) in the indicated groups at 7 and 30 days after MI (n = 6 mice per experimental group; $^{*}P < 0.05$ vs IKKα$^{flox/flox}$; $^{\#}P < 0.05$ vs mIKKα$^{-/-}$). (**E**) Representative images of the HE staining and quantitative analysis of the infarct area/left ventricular (LV) area and wall thickness in IKKα$^{flox/flox}$ and mIKKα$^{-/-}$ at 3, 7, and 30 days after MI (n = 4 mice per experimental group, ×50; $^{*}P < 0.05$ vs IKKα$^{flox/flox}$; $^{\#}P < 0.05$ vs IKKα$^{flox/flox}$ MI 7 days; $^{\dagger}P < 0.05$ vs IKKα$^{flox/flox}$ MI 30 days). EF, ejection fraction; FS, fractional shortening; HE, hematoxylin-eosin; IVSd, interventricular septum diastole; LVIDd, left ventricular internal dimension diastole.

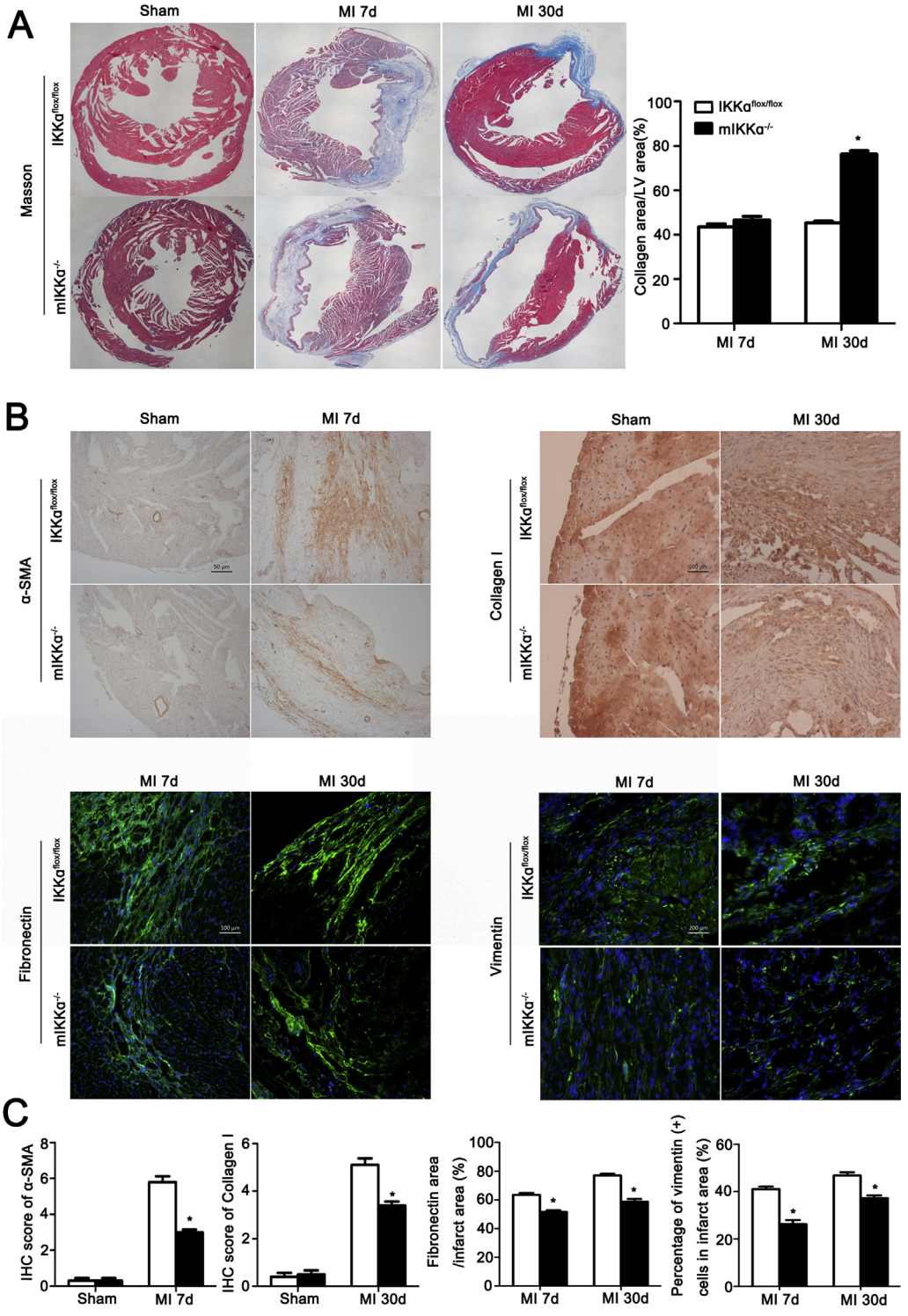

Figure 3. Macrophage IκB kinase α (IKKα) deficiency delays angiogenesis, collagen deposition, and fibrosis after myocardial infarction (MI). (**A**) Representative images of Masson staining and quantitative analysis of the collagen area/left ventricular (LV) area in IKKα$^{flox/flox}$ and macrophage-specific IKKα knockout (mIKKα$^{-/-}$) at 3, 7, and 30 days after MI (n = 4 mice per experimental group, ×50; *$P < 0.05$ vs IKKα$^{flox/flox}$) (**B**) Representative immunohistochemistry and immunofluorescent staining images showing expression of α-SMA, collagen I, fibronectin, and vimentin in the infarct area of myocardial tissue sections at 7 or 30 days after MI (n = 4 mice per experimental group; α-SMA: ×100, collagen I: ×400; fibronectin: ×200, vimentin: ×400). (**C**) Semiquantitative analysis of each factor (*$P < 0.05$ vs IKKα$^{flox/flox}$). α-SMA, alpha-smooth muscle actin.

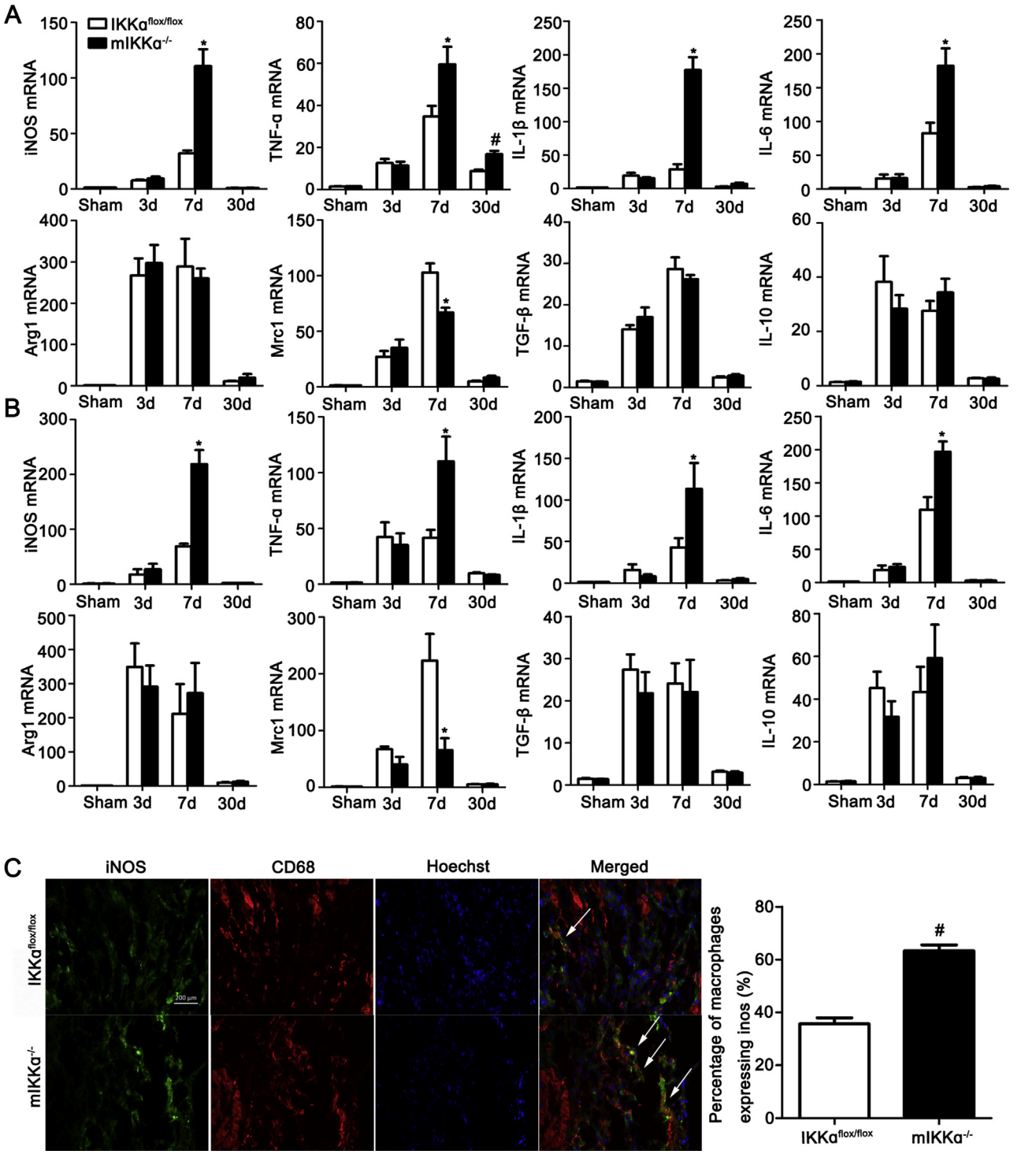

Figure 4. Macrophages lacking IκB kinase α (IKKα) regulates macrophage polarizing to the M1 phenotype at 7 days after myocardial infarction (MI). (**A**) Relative levels of mRNA of proteins representing the M1 (iNOS, tumor necrosis factor-α [TNF-α], interleukin-1β [IL-1β], and IL-6) or M2 (arginase 1 [Arg1], Mrc-1, transforming growth factor-β [TGF-β], and IL-10) phenotype in the infarct area at 3, 7, and 30 days after MI (n = 4 mice per experimental group, $*P < 0.05$ vs IKKα$^{flox/flox}$, $^{\#}P < 0.05$ vs IKKα$^{flox/flox}$ and macrophage-specific IKKα knockout (mIKKα$^{-/-}$) sham, 3 and 7 days). (**B**) Relative levels of mRNA for proteins representing the M1 or M2 phenotype in a border area at 3, 7, and 30 days after MI (n = 4 mice per experimental group, $*P < 0.05$ vs IKKα$^{flox/flox}$). (**C**) Representative images of coimmunofluorescent staining showing iNOS (**green**) and CD68 (**red**) in myocardial tissue sections of mIKKα$^{-/-}$ and IKKα$^{flox/flox}$ at 7 days after MI (**arrows** indicate cells expressing both iNOS and CD68; n = 3 independent experiments, ×400). The quantitative analysis showed the rate of macrophages expressing iNOS in the myocardium of mIKKα$^{-/-}$ and IKKα$^{flox/flox}$ at 7 days after MI ($*P < 0.05$ vs IKKα$^{flox/flox}$). iNOS, inducible nitric oxide synthase.

factors and M1 markers (TNF-α, IL-1β, IL-6, and inducible nitric oxide synthase [iNOS]) were higher in mIKKα$^{-/-}$ in the infarct zone (Fig. 4A) and border zone (Fig. 4B) than in IKKα$^{flox/flox}$ at 7 days after MI. In addition, the amount of M2 markers (Mrc1) was lower in mIKKα$^{-/-}$ than in IKKα$^{flox/flox}$ at 7 days. However, there are no significant differences in the pro- and anti-inflammatory factors as well as the M1 or M2 markers between mIKKα$^{-/-}$ and IKKα$^{flox/flox}$ at 3 days after MI. At 30 days after MI, the expression levels of both pro- and anti-inflammatory factors were much lower than 3 and 7 days with no obvious differences in mIKKα$^{-/-}$ and IKKα$^{flox/flox}$, except for a higher expression of TNF-α in mIKKα$^{-/-}$ than IKKα$^{flox/flox}$ in the infarct zone. This result means that macrophage polarization can mainly be regulated by IKKα at 7 days after MI. Next, the immunofluorescence colocalization of iNOS and CD68 showed more macrophages expressing iNOS in mIKKα$^{-/-}$ than IKKα$^{flox/flox}$ at 7 days after MI (Fig. 4C). In conclusion, macrophage deficiency of IKKα facilitates easier polarization to the M1 phenotype and secretion of proinflammatory factors than normal macrophages at 7 days after MI.

IKKα deficiency in macrophages inhibited the NF-κB/RelB pathway at both 3 and 7 days after MI

IKKα is one of the regulating kinases in the NF-κB pathway, especially in the alternative pathway. We tested the activation of both classical and alternative pathways of NF-κB with western blots at 3, 7, and 30 days. We observed that the expression of phos-RelB was lower in mIKKα$^{-/-}$ than IKKα$^{flox/flox}$ at both 3 and 7 days after MI (Fig. 5). However, the expression of phos-p100/p52, which is the subset of the dimer composed of RelB in the alternative pathway, showed no differences in both groups. Similarly, the analysis of IκBα, phos-IκBα, p65, and p-p65, which represent the classical pathway, did not display any observable differences. In addition, the expression of the NF-κB pathway showed no significant differences between the sham and MI 30 days group as well as mIKKα$^{-/-}$ and IKKα$^{flox/flox}$ (Supplemental Fig. S2A). These findings reveal that IKKα deficiency may inhibit RelB in the NF-κB alternative pathway and result in macrophage polarization to the M1 phenotype after MI.

IKKα deficiency in macrophages enhanced the MEK1/2/ERK1/2 pathway at both 3 and 7 days after MI

There is growing evidence that the MAPK pathway is one of the important pathways regulating cellular events and the inflammatory response after MI.[19] Therefore, we sought to investigate whether IKKα had a role in interfering with MAPK cascade activation. The immune blotting revealed that the expressions of phos-MEK1/2 and phos-ERK1/2 were higher in mIKKα$^{-/-}$ than IKKα$^{flox/flox}$ at both 3 and 7 days after MI (Fig. 6). However, there were no significant differences between the 2 groups for phos-JNK, phos-p38, JNK, and p38. Moreover, expression of the MAPK pathway showed no significant differences between the sham and MI 30 days group as well as mIKKα$^{-/-}$ and IKKα$^{flox/flox}$ (Supplemental Fig. S2B). Our results demonstrate that IKKα deficiency will induce macrophage polarization to the M1 phenotype after MI by largely enhancing the MEK1/2/ERK1/2 pathway in hearts on MI.

Discussion

In this study, we identified IKKα as a positive regulator during the procedure for macrophage polarization after MI. We demonstrated that these mice would have severe cardiac dysfunction, attenuated ventricular remodelling angiogenesis, and fibrosis after MI when there is a deficiency of IKKα in macrophages. Strikingly, this protective influence of IKKα was attributed to its regulation of macrophage polarization. We found the macrophages lacking IKKα were easier to polarize to the M1 phenotype at 7 days after MI. Mechanistically, IKKα-regulated macrophage polarization mostly depended on the inhibition of the NF-κB/RelB pathway and activation of the MEK1/2/ERK1/2 pathway.

The initiation and resolution of postinfarction inflammation determine the extent of cardiac injury and the progress of cardiac repair.[20] At baseline, no significant differences were observed between mIKKα$^{-/-}$ and IKKα$^{flox/flox}$ in terms of LV structure and function. The mortality of mIKKα$^{-/-}$ after MI was much higher than IKKα$^{flox/flox}$, and the animals died mostly between 5 and 10 days. Although echocardiographic analysis at 7 days showed no differences between 2 groups, HE and Masson trichrome staining indeed showed obvious differences in fibrosis and collagen deposition in the infarcted ventricular wall. That was the start of the differences that would arise between the 2 groups. At 30 days, the surviving mIKKα$^{-/-}$ showed more severe cardiac dysfunction and scar formation. The main cause of death was left ventricle rupture, which was associated with the weakening of tensile strength. The low survival rate of mIKKα$^{-/-}$ may be due to limited collagen deposition and a thin ventricular wall. As we know, troponin-T, CK-MB, and LDH start to elevate a few hours after MI and return to normal about few days or a week in humans. In Supplemental Figure S1A, we observed that troponin-T was highly expressed at 3 days after MI, with a difference occurring between the 2 groups at 30 days, whereas CK-MB and LDH were highly expressed at 30 days. However, we selected only 3 time points after mice MI, and mice may have some differences in MI markers compared with humans.

Ventricular remodelling will sustain for a long time until there is a counterbalance between distending forces and tensile strength in collagen scar. α-SMA is an important marker for angiogenesis that is responsible for the level of fibrosis during ventricular remodelling after MI. The expression of α-SMA in mIKKα$^{-/-}$ was significantly lower than that in IKKα$^{flox/flox}$, which indicated that angiogenesis of mIKKα$^{-/-}$ at 7 days after MI was much lower than that in IKKα$^{flox/flox}$. Consequently, the fibrosis level of IKKα$^{flox/flox}$ was higher than that of mIKKα$^{-/-}$ with high expression levels of collagen, fibronectin, and vimentin. These phenotypes demonstrated that mIKKα$^{-/-}$ had attenuated fibrosis, scar formation, and ventricular remodelling after MI. It seems confusing that there was a large collagen area and a low immunohistochemistry (IHC) score for collagen I in mIKKα$^{-/-}$ 30 days after MI. However, these results are not contradictory because the hearts of mIKKα$^{-/-}$ at 30 days after MI had large infarct collagen areas but also a low collagen density. The low collagen density is the reason for the large infarct area, thin wall thickness, and failure of ventricular remodelling.

Although many studies have showed functions for macrophages in infarcted hearts, our knowledge about these

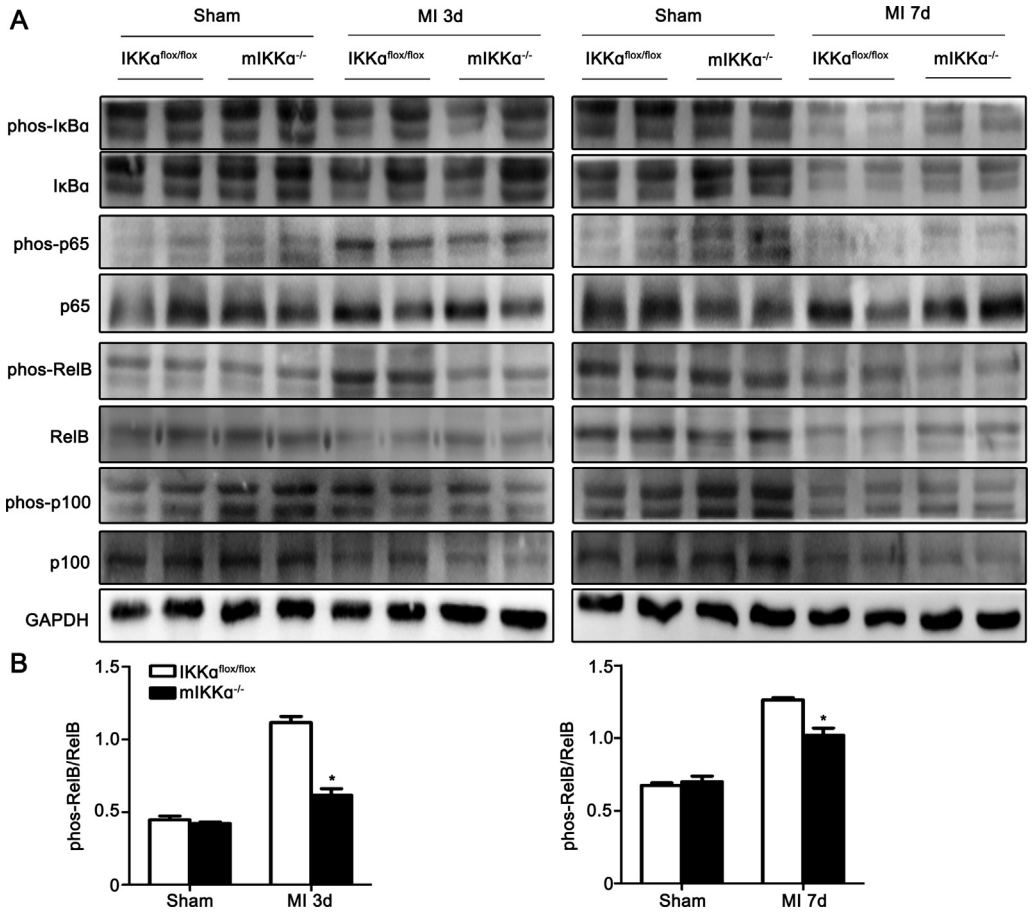

Figure 5. Macrophage IκB kinase α (IKKα) deficiency regulates macrophage polarization by inhibiting the nuclear factor-κB (NF-κB)/RelB pathway. (**A, B**) Representative western blots and quantitative results showing the phosphorylation and total protein of IκBα, p65, RelB, and p100/p52 of the NF-κB pathway in the heart tissue of macrophage-specific IKKα knockout (mIKKα$^{-/-}$) and IKKα$^{flox/flox}$ at 3 and 7 days after myocardial infarction (MI) (n = 6 independent experiments; *$P < 0.05$ vs IKKα$^{flox/flox}$).

macrophages is still limited.[17,21,22] Macrophages have 2 distinct subsets, including M1 and M2. M1 macrophages can make an effective immune response to some pathogens and result in an excessive destruction in tissue. In contrast, M2 macrophages can establish homeostasis and facilitate tissue repair with its potent anti-inflammatory role.[23,24] Previous studies show a proinflammatory result for macrophage deficiency in IKKα.[14-16] In our study, macrophages accumulated to the infarcted heart at 3 days after MI and reduced to near normal levels at 30 days after MI, whereas they displayed adverse polarization phenotypes at 7 days. The expression of proinflammatory factors and M1 markers (TNF-α, IL-1β, IL-6, and iNOS) were much higher, whereas the M2 marker (Mrc1) was lower in mIKKα$^{-/-}$ than in IKKα$^{flox/flox}$ at 7 days after MI in both the infarct zone and border zone, which demonstrated that macrophages were easier to polarize to the M1 phenotype in mIKKα$^{-/-}$ than in IKKα$^{flox/flox}$. The reason for some nondifferential inflammatory factors (Arg1, IL-10, and transforming growth factor-β) may be explained by a compensatory response in the complex inflammatory reaction after MI. The nonconformity of differences in molecular pathways and lack of differences in inflammatory markers between the 2 groups at 3 days after MI should be explained by the time it took for changes in pathways to emerge as phenotypes. The very beginning of differences in pathways between the 2 groups occurred at 3 days after MI, and the differences in phenotypes arose at 7 days.

NF-κB is an essential transcription factor, especially in the inflammatory response. The activation of the classical pathway relies on the phosphorylation of the IKK complex, which consisted of 2 catalytic subunits for IKKα, including IKKβ and IKKγ. IKKβ is the main kinase-activating classical pathway. IKKβ was shown to play a protective role in ventricular remodelling at 28 days after MI.[25] In this study, we focused on the role of IKKα in macrophages during the inflammatory stage after MI. The IKK complex will induce degradation of IκBα after it is phosphorylated. The activated IκBα induces phosphorylation of p65 or RelA to participate in transcription. Consistently, IKKα is the only kinase that activates the alternative pathway. It will induce the phosphorylation of RelB or p100 for transcription. Consistent with previous studies,[26,27] our findings revealed that phos-RelB was decreased in mIKKα$^{-/-}$ at both 3 and 7 days after MI. The activation of the NF-κB pathway was almost normal at 30 days after MI. We hypothesize that IKKα is a protective kinase of RelB that maintains the stability of macrophage

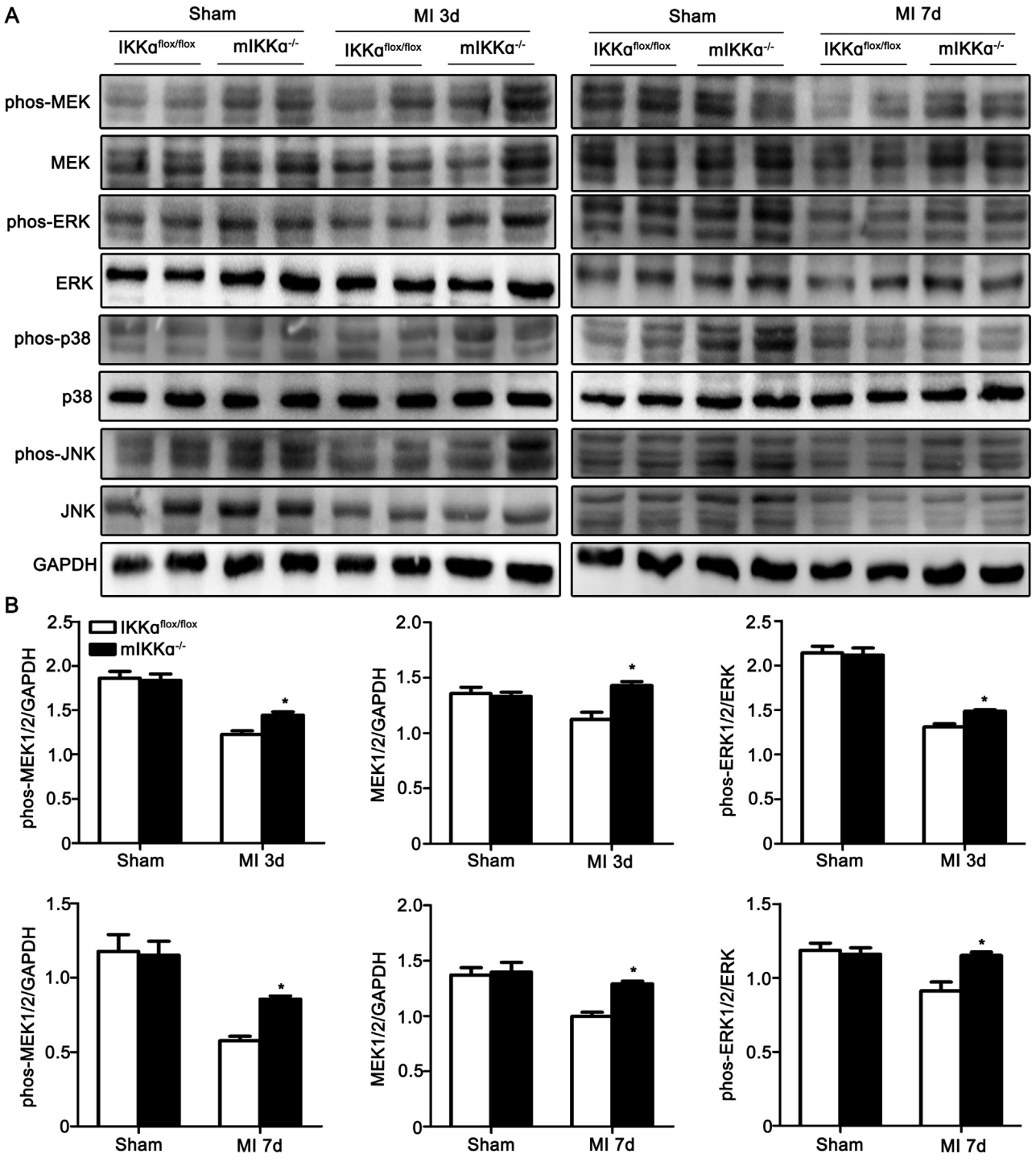

Figure 6. Macrophage IκB kinase α (IKKα) deficiency regulates macrophage polarization by activating the MEK/ERK pathway. **(A, B)** Representative western blots and quantitative results showing the phosphorylation and total protein of MEK1/2, ERK1/2, JNK, and p38 of the MAPK pathway in the heart tissue of macrophage-specific IKKα knockout (mIKKα$^{-/-}$) and IKKα$^{flox/flox}$ at 3 and 7 days after myocardial infarction (MI) (n = 6 independent experiments; *$P < 0.05$ vs IKKα$^{flox/flox}$).

polarization by inhibiting the tendency of macrophages to polarize to the M1 phenotype.

Several studies have confirmed a strong relationship between the changes in myocardial MAPK activation and the process of post-MI ventricular remodelling.[28] There were some studies that reported that blocking p38 in macrophages could decrease iNOS expression.[29,30] However, our results showed a significantly increased expression of phos-MEK1/2 and phos-ERK1/2 in mIKKα$^{-/-}$ at both 3 and 7 days after MI. The activity of the MAPK pathway decreased to natural levels at 30 days after MI. Previous studies have revealed that IKKs is an upstream kinase regulating MEK/ERK through the

IKK-TPL-2-MEK/ERK pathway.[31,32] We made the hypothesis that IKKα deficiency in macrophages regulates macrophage polarization to the M1 phenotype through activating the MEK1/2/ERK1/2 pathway, which means that IKKα is an inhibitor of MEK1/2/ERK1/2 in a proinflammatory reaction.

Limited by time, this study only performed the research *in vivo*, and the significant differences in the phenotypes between mIKKα$^{-/-}$ and IKKα$^{flox/flox}$ after MI revealed a critical role for IKKα in regulating macrophage polarization in heart tissue during the acute inflammatory response after MI. We will devote further time to researching the function of IKKα in macrophages *in vitro* and adapt the experiments *in vivo* for clinical use. In this regard, further studies are required to validate these mechanisms.

Conclusions

Murine macrophage deficiency of IKKα promotes polarization to the M1 phenotype at 7 days after MI by inhibiting the NF-κB/RelB pathway and enhancing the MEK1/2/ERK1/2 pathway, which results in a severe inflammatory reaction, cardiac dysfunction, reduced fibrosis, collagen deposition, and delayed scar formation. Consequently, this study also provides novel insights into the molecular mechanisms of cardiac remodelling after MI. Based on these findings, our observations may help to develop novel therapeutic strategies for the treatment of MI.

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Disclosures

The authors have no conflicts of interest to disclose.

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Supplementary Material

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