Expression and subcellular localization of MK5, ERK3 and ERK4 in cardiac fibroblasts and myocytes

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Introduction. MAP kinase-activated protein kinase-5 **(**MK5, PRAK) was originally discovered as target of p38 MAPK. Subsequent studies suggested that MK5 activity is regulated by atypical MAPKs ERK3 and ERK4. The physiological role of MK5, in addition to the mechanisms regulating its activity and subcellular localization, remain controversial. MK5 mRNA is highly expressed in heart. Both ERK3 and MK5 haploinsufficient mice show reduced cardiac collagen expression following pressure overload induced by constriction of the transverse aorta. Furthermore, scar rupture was more frequent in MK5 haploinsufficient mice following myocardial ischemia induced by ligation of the left anterior descending coronary artery. Thus, ERK3-MK5 signalling may play a role in fibrosis. The present study was to determine the expression and subcellular localization of ERK3, ERK4, and MK5 in cardiac fibroblasts and myocytes.

Methods. Cardiac fibroblasts were isolated from $MK5^{+/+}$ and $MK5^{-/-}$ mice. Subconfluent cultures of fibroblasts from passages 0, 1, 2 and 3 were used. Proteins and RNA were also prepared from freshly isolated adult mouse cardiac myocytes. Protein and mRNA levels were determined by immunoblot and quantitative real-time PCR, respectively. The subcellular localization of ERK3 and MK5 was determined by immunocytofluorescence and confocal microscopy.

Results.MK5 and ERK3 immunoreactivity was detected in fibroblasts but negligible in myocytes. In contrast, ERK4 immunoreactivity was detected in myocytes. The abundance of MK5 mRNA in fibroblasts and myocytes was similar. In contrast, ERK3 and ERK4 mRNA was more abundant in myocytes. Confocal microscopy revealed that, in fibroblasts, MK5 and ERK4 immunoreactivity localized to the nucleus whereas ERK3 immunoreactivity localized to the cytoplasm. A similar pattern of subcellular distribution was observed in passage numbers 0-3. ERK3 immunoreactivity and subcellular localization was similar in fibroblasts from $MK5^{+/+}$ and $MK5^{-/-}$mice. In addition ERK3 immunoreactivity was unaffected by the acute knockdown of MK5 using siRNA. In contrast, ERK4 immunoreactivity was reduced in fibroblasts from $MK5^{-/-}$mice.

Discussion. In actively dividing cardiac fibroblasts, MK5 and ERK4 were observed in the nucleus whereas ERK3 was cytoplasmic. Furthermore, in contrast to other cell systems, in cardiac fibroblasts ERK3 was not destabilized by the absence of MK5, suggesting the possible presence of an unidentified binding partner in these cells. Reduced levels of ERK4 immunoreactivity in $MK5^{-/-}$fibroblasts suggests a role for MK5 in determining the expression or stability of ERK4 in these cells. Interestingly, in spite of having comparable amounts of MK5 mRNA and a 2-fold greater abundance of ERK3 mRNA, MK5 and ERK3 immunoreactivity was negligible in myocytes. In contrast, ERK4 immunoreactivity was greater in myocytes. These observations suggest ERK3, ERK4, and MK5 play cell specific roles in the heart.

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