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Transient Up-regulation of Myotonic Dystrophy Protein Kinase-binding Protein, MKBP, and HSP27 in the Neonatal Myocardium

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ABSTRACT. **Myotonic dystrophy protein kinase (DMPK)-binding protein, MKBP, has high homology with a small heat shock protein, HSP27. Western blotting analyses showed that MKBP level in rat heart rapidly increased, with a sharp peak at one week after birth (3-fold the level at the fetus), but that it rapidly decreased (1/10 of peak value at 13 weeks). Human myocardium also showed similar age-dependency. Similar but small increase of HSP27 was observed in the neonatal rat myocardium, but not in constitutive and inducible forms of HSP70. Immunofluorescence analysis localized MKBP at the Z lines and intercalated discs in the rat myocardium. MKBP may protect actin cytoskeleton or other proteins of heart muscle against oxidative stress in the neonate.**

Key words: myotonic dystrophy protein kinase-binding protein/heat shock protein 27/myocardium/age-dependency/oxidative stress

Recently, we have discovered myotonic dystrophy protein kinase (DMPK)-binding protein (MKBP) in a human skeletal muscle cDNA library using a yeast two-hybrid system (1). MKBP enhances DMPK activity and protects it against heat inactivation (1). MKBP shows high homology with a small heat shock protein HSP27 (1) that is thought to protect actin filaments against heat, oxidative, and mechanical stresses in muscle cells (2–4). Here we show the sharp and transient upregulation of MKBP and HSP27 in the neonatal myocardium of the rat and human and the localization of MKBP at Z lines and intercalated discs.

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Abbreviations: MKBP, myotonic dystrophy protein kinase-binding protein; HSP27, heat shock protein 27.

Materials and Methods

Antibodies

Anti-MKBP polyclonal antibody (C2) was generated against the GST-MKBP fusion protein (1). Goat anti-human HSP27, constitutive and inducible forms of HSP70 and horseradish peroxidase (HRP)-conjugated anti-goat IgG were obtained from Santa Cruz Biotechnology, rhodamine B-conjugated anti-rabbit IgG, fluorescein-conjugated anti-mouse IgG from Amersham, monoclonal anti-desmin antibody from Sigma, and monoclonal anti-glyceralaldehyde-3-phosphate dehydrogenase (G3PDH) antibody from Chemicon International.

Samples

All animal experiments followed a protocol approved by the Ethical Committee for Animal Experimentation at Yamaguchi University School of Medicine and were within the committee guidelines for animal experimentation. To examine the effect of postmortem interval, some rats were kept at 20°C for 14 hours. The ventricular heart muscles of Wistar rats (n=13 for fetus 20 days after expected gestation, n=11 for neonate, n=6 for one week, n=3 each for 4, 8, and 13 weeks) were excised after injection of pentobarbital over-

dose. Human heart samples (n=20), within half day post-mortem, were obtained at autopsy under informed consent. The samples were quickly frozen and kept at −70°C until analyses. Hearts of the same age were combined for homogenization, and subcellular fractionation was performed as previously described (5, 6); the 1,000×g pellet, 100,000×g pellet and its supernatant of the homogenate were designated as the P1, P2, and S fractions, respectively. The protein was determined by the method of Lowry *et al.* (7).

Electrophoresis and immunoblotting

Samples were electrophoresed in 12.5% polyacrylamide gels by the method of Laemmli (8) and immunoblotted by the method of Towbin *et al.* (9), using either anti-MKBP, anti-HSP27, anti-HSP70 (constitutive, inducible), or anti-G3PDH antibody. Proteins were visualized with the ECL Western blotting detection kit (Amersham Co.), quantified by an image analyzer (Densitograph AE-6900, Atto), and expressed as an arbitrary unit/fixed amount of protein, as described previously (5, 6).

Immunofluorescence microscopy

Hearts of rats (8 weeks old) were immersed in OCT compound (Miles) and rapidly frozen in liquid nitrogen. After sectioning at 6 μm in thickness, the specimens were fixed on coverslips with acetone-methanol, and were double stained with anti-desmin and anti-MKBP antibodies followed by fluorescein-conjugated anti-mouse IgG and rhodamine-conjugated anti-rabbit IgG, respectively, as previously reported (1, 10).

Results and Discussion

High level of MKBP in rat and human heart in the neonate

In rat heart, MKBP level rapidly increased, with a

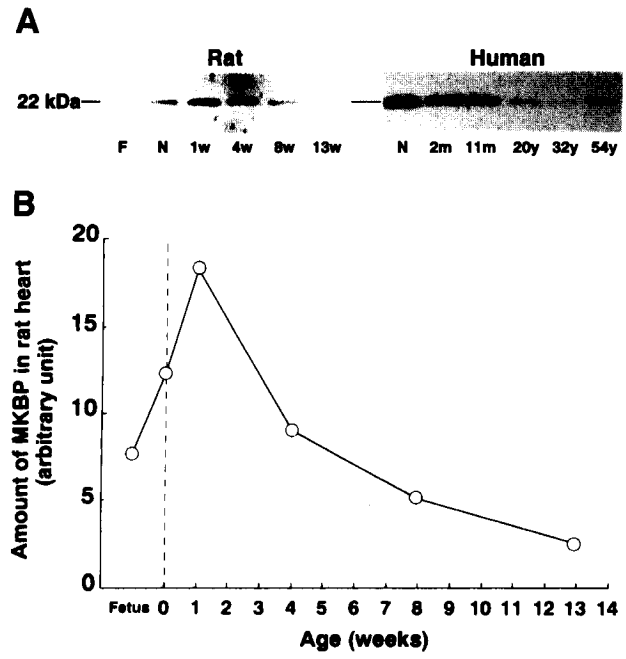

Fig. 1. Age-dependent expression of MKBP in the rat and human heart muscle. Panel A is the immunoblot showing the age-dependency; panel B shows the quantification of the blot (the level expressed in an arbitrary unit). An equal amount of homogenate protein (15 μg/lane) was applied to the gels.

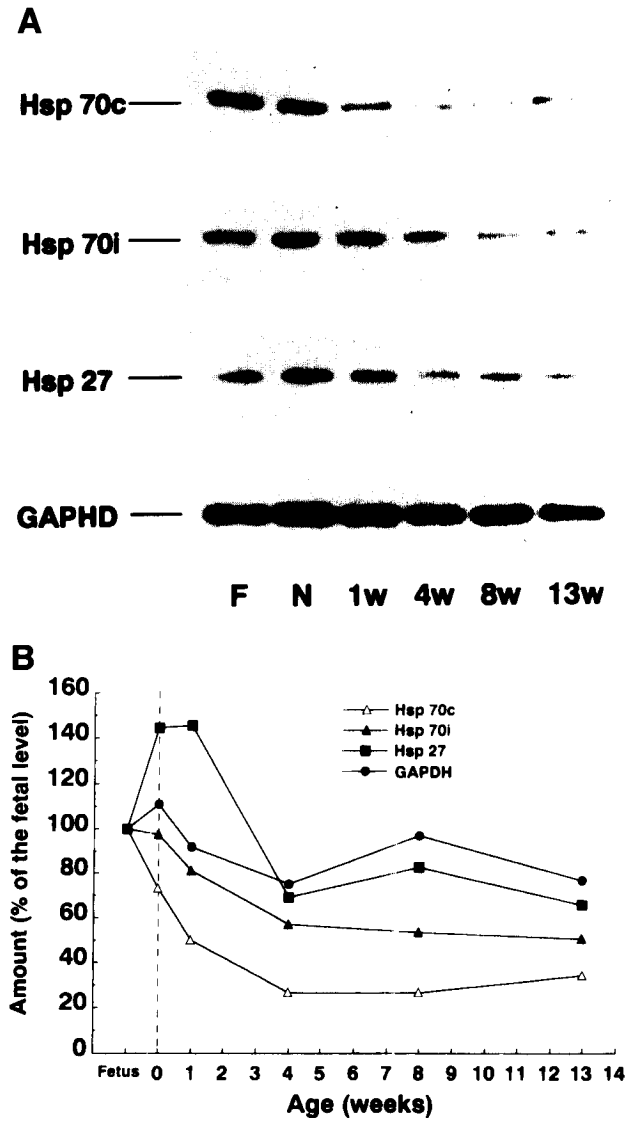

Fig. 2. Age-dependent expression of HSP27, constitutive and inducible forms of HSP70 and G3PDH in the rat heart. Panel A is the immunoblots; panel B shows the quantification of the blot (the level of proteins expressed in an arbitrary unit). An equal amount of homogenate protein (10 μg/lane) was applied to the gel.

peak at one week after birth (3-fold of fetus level), and decreased rapidly (1/10 of peak level at 13 weeks after birth) (Fig. 1). In the human heart, MKBP level was also high in neonatal heart, with sharp decrease with age (2–11 months), and was low between the ages of 11 to 69 years. Postmortem interval (14 hours) did not affect MKBP level in rat heart (data not shown). Similarly but much less markedly, the MKBP level in the rat skeletal muscle increased transiently in neonate (data not shown).

HSP27 also shows high levels in the neonate

HSP27 expression shows a similar pattern of age-dependency as MKBP, although the increase in neonate was small with a rapid decrease thereafter (Fig. 2). By contrast, there was no increase in neonate in constitutive and inducible forms of HSP70 or G3PDH (well known for constitutive expression).

Distribution of MKBP

Subcellular fractionation shows an abundance of MKBP in the S (cytosolic, 68.7%) and P1 (nucleus-myofibril, 24.7%) fractions. Triton X-100 extracts nuclear components and leaves myofibril or cytoskeletal components in the residue. However, 97.5% of MKBP in the P1 fraction was distributed to the residue, suggesting its predominance in the myofibril. The increase in MKBP at one week postnatum was attributed to the increase in cytosolic (S) fraction (about 2.4-fold of fetus and immediately after birth). We reported that MKBP is localized at Z lines and neuromuscular junction in the skeletal muscle (1), and that desmin is localized at Z lines and intercalated discs in rat heart (10). In this study, immunofluorescence analysis showed for the first time that MKBP is localized at the intercalated discs of the heart in addition to the Z lines in the rat heart (Fig. 3). Desmin co-localized with MKBP. The antigen preadsorption diminished the MKBP staining, confirming antibody specificity (data not shown).

Table I. DISTRIBUTION OF MKBP IN SUBCELLULAR FRACTIONS

	P1		P2	S
MKBP (24 kDa)	24.7		6.6	68.7
	Res.	Ext.		
	24.1	0.6		

The amount of MKBP in the different fractions was determined as described in "*Materials and Methods*" and expressed as % of the total amount.

Physiological implication of MKBP up-regulation in neonatal heart

The neonatal heart is exposed to hypoxia during labor, followed by exposure to increased O_2 pressure after onset of self-respiration after birth. Additionally, O_2 consumption of the myocardium increases as a result of enhanced contraction after birth. This physiological hypoxia-reoxygenation would expose the myocardium to oxidative stress, as does myocardial ischemia-reperfusion (5, 6). On the other hand, HSP27 protects actin cytoskeleton against heat, oxidative and mechanical stresses (2, 3). Because MKBP and HSP27 belong to the small heat shock protein family (1), the up-regulation of MKBP and HSP27 may be an adaptive response to protect actin cytoskeleton in the Z lines and intercalated discs against oxidative stress evoked by physiological hypoxia-reoxygenation. Alternatively, the observation that postnatal increase in MKBP is predominantly cytosolic suggests that MKBP may protect actin or

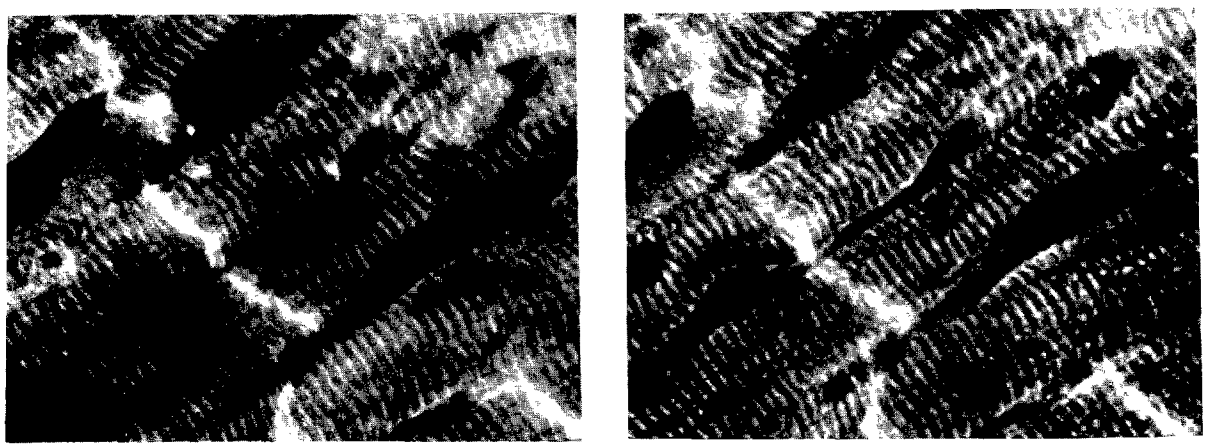

Fig. 3. Immunofluorescence localization of MKBP and desmin. MKBP (left) and desmin (right) shows the co-localization at the Z lines and intercalated discs.

other proteins that are rapidly synthesized in the ribosome during postnatal proliferation. Furthermore, MKBP may also play a role as a chaperone or a regulator of dystrophy kinase, whose physiological role remains elusive.

In conclusion, we found transient but striking up-regulation of myocardial MKBP in the neonatal myocardium, which may be involved in the protection against ischemia-reperfusion injury of the heart.

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