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Essential role of *Hand2* in interventricular septum formation and trabeculation during cardiac development

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Abstract

Interventricular septum (IVS) formation is one of the key events in the development of a four-chambered heart. We previously showed that the basic helix–loop–helix transcription factor *Hand1* plays an important role in the formation of the IVS. Here, we found that the other *Hand* gene, *Hand2*, regulated expansion, trabeculation, and IVS formation in the embryonic heart. In transgenic embryos expressing *Hand2* in the whole ventricles, the boundary region between the left and right ventricles expanded outwards, resulting in complete absence of the IVS. Moreover, trabecular formation was observed even in a region where the IVS was expected to form. In some transgenic embryos with heterogeneous expression of the transgene, a muscular septum did not form in a region where *Hand2* was expressed, but an incomplete septum was identifiable in a region where *Hand2* was not expressed, suggesting that septum formation was strictly regulated by the expression domain of *Hand2*. Furthermore, expression of trabecular markers including *ANF*, *BNP*, and *connexin40* was significantly up-regulated in the ventricles of *Hand2* transgenic embryos as well as in H9c2 cells over-expressing *Hand2*. These results suggested that the absence of *Hand2* expression in the interventricular boundary region inhibits expansion and trabeculation in this area, contributing to the proper formation of the IVS.

Keywords: *Hand2*; Interventricular septum; Trabeculation; Cardiac development; Transcription factor

Separation of pulmonary and systemic circulation was a key event during evolution of the cardiovascular system. A complete ventricular septum in birds and mammals improved the efficiency of gas exchange and enabled elevated systemic pressures without elevated pulmonary pressures, providing adaptations for endurance activities and active life styles in these species [1]. However, molecular mechanisms for interventricular septum (IVS) formation remain largely unknown.

Hand2/dHAND is a basic-helix–loop–helix transcription factor. Targeted disruption of *Hand2* severely impaired development of the right ventricle (RV), outflow tract, and aortic arch arteries, indicating that *Hand2* was indispensable for the formation of the cranial elements of the developing heart [2]. However, its precise function in the morphogenesis of the cardiac chambers and septa remains to be elucidated. Our previous work showed that the other *Hand* gene, *Hand1/eHAND*, expressed predominantly in the outer curvature of the left ventricle (LV), regulated expansion of the outer curvature and that absence of *Hand1* expression in the boundary region between the left and right ventricles was critical for IVS formation [3]. Recently, McFadden et al. [4] generated cardiac-specific *Hand1* knock-out mice and reported that *Hand1* and *Hand2* regulated expansion of the ventricles in a gene dose-dependent manner. These results suggested that *Hand2*, in concert with *Hand1*, may regulate IVS formation.

To investigate the function of *Hand2* during cardiac morphogenesis, we created transgenic mice expressing *Hand2* under the control of the *beta-myosin heavy chain*

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(β-MHC) promoter [5]. Transgenic expression of *Hand2* in the whole ventricles caused expansion of the outer curvature of the interventricular boundary region, resulting in complete absence of IVS formation. Interestingly, in some transgenic embryos showing heterogeneous expression of the transgene, partial formation of the IVS was observed in a region where *Hand2* was not expressed, suggesting that IVS formation may be tightly regulated by the expression domain of *Hand2*.

Materials and methods

Generation of transgenic mice. The mouse full-length *Hand2* cDNA was synthesized by reverse transcription-polymerase chain reaction (RT-PCR). A deletion mutant of *Hand2* (*Hand2-ΔHLH*) was generated by removing the helix–loop–helix domain (amino acids 112–154) using PCR-based mutagenesis. The identity was confirmed by DNA sequencing. The *β-myosin heavy chain* (*MHC*) promoter [5] (kindly provided by Jeffrey Robbins, Children's Hospital Research Foundation, Cincinnati) was ligated to Myc-tagged *Hand2* or FLAG-tagged *Hand2-ΔHLH* with the human growth hormone poly(A) signal sequence. To create transgenic mice over-expressing *Hand2* in the RV, the rat *myosin light chain 2V* (*MLC2V*) promoter [3,6] was ligated to Myc-tagged *Hand2* with the human growth hormone poly(A) signal sequence. The creation of transgenic mice was done in a standard manner [7]. F0 embryos were dissected at E9.5, E10.5, E11.5, and E12.5, and genotyping was performed using PCR on DNA isolated from the yolk sacs or placenta. PCR primer pairs used for detection of *β-MHC-Hand2* and *β-MHC-Hand2-ΔHLH* were 5′-GAGAAGGGTAAACTCCTGAGTGCTG-3′ and 5′-ATAAGCCAG CCGTGGAAGTAGG-3′, and for detection of *MLC2v-Hand2* were 5′-T CCTCCTCTTCCTCCCCCTC-3′ and 5′-ATAAGCCAGCCGTGGAA GTAGG-3′. All animal procedures were approved by the Animal Research Committee, Graduate School of Medicine, Kyoto University.

In situ hybridization. In situ hybridization was performed as described previously [8]. Briefly, embryos were fixed in 4% paraformaldehyde at 4 °C overnight, dehydrated through graded ethanol and xylene, and embedded in paraffin wax. Sections of 6-μm thickness were hybridized with ^{35}S-CTP labeled riboprobe at 55 °C overnight. After hybridization, they were treated with RNase A, washed, and dehydrated through graded ethanol, and emulsion autoradiography was performed. Probes for *atrial natriuretic factor* (*ANF*), *brain natriuretic peptide* (*BNP*), *Hand1*, *Hand2*, and *Tbx 5* were described previously [3,8]. The probe for *Hand2* (nucleotides 292–644) can hybridize to endogenous *Hand2* as well as the wild type and mutant *Hand2* transgene. Probes for *BMP10* (nucleotides 188–749), *versican*/*Cspg2* (nucleotides 6902–7374), and *connexin40* (*Cx40*) (nucleotides 147–1126) were synthesized by RT-PCR. The identity of the probes was confirmed by DNA sequencing.

Immunohistochemistry. Immunohistochemistry for detection of FLAG-tagged Hand2-ΔHLH was performed as described previously [3]. Immunohistochemistry for detection of Myc-tagged Hand2 using Histofine mouse stain kit was performed according to the manufacturers' protocol (Nichirei, Tokyo, Japan). Briefly, paraffin sections of embryos were deparaffinized, pretreated with 0.025% subtilisin (Sigma, St. Louis, MO), blocked using Blocking Reagent A (Nichirei, Tokyo, Japan) and incubated with an anti-Myc monoclonal antibody (Sigma, St. Louis, MO) (1:200) overnight at 4 °C. Sections were then blocked using Blocking Reagent B (Nichirei, Tokyo, Japan) and incubated with Simple Stain Mouse MAX-PO (Nichirei, Tokyo, Japan). Peroxidase activity was detected with 3,3′-diaminobenzidine.

Cell culture, construction of expression vectors, and transfection. H9c2 cells were purchased from American Type Culture Collection (CRL-1446). Cells were maintained in Dulbecco's modified Eagle's medium with 4.5 g/L glucose supplemented with 10% fetal calf serum. The *SRα* promoter, a hybrid promoter comprising the simian virus 40 early region promoter and the R region of the HTLV-1 long terminal repeat [9], was ligated to *Hand2* or *Hand2-ΔHLH* with the human growth hormone poly(A) signal sequence. The empty vector was used as a mock. The expression vectors were transfected into H9c2 cells using FuGENE6 Transfection Reagent (Roche, Indianapolis, IN).

Quantitative real-time polymerase chain reaction. Total RNA was isolated from H9c2 cells using Trizol reagent (Invitrogene, Carlsbad, CA) and cDNA was synthesized using Superscript III reverse transcriptase (Invitrogene, Carlsbad, CA). Oligonucleotide primers were designed using Primer Express 2.0 software (Applied Biosystems, Foster city, CA). Relative quantification of gene products was performed using the SYBR Green PCR Master Mix (Applied Biosystems, Foster city, CA) and the ABI Prism 7900HT sequence detection system (Applied Biosystems, Foster city, CA). Quantification of the glyceraldedehyde-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control to normalize for differences in the amount of total cDNA present in each sample. To calculate the fold of activation, the average data were divided by those obtained from vector-only transfected cells. Primer sequences are provided in the supplement, available online. Data were analyzed by two-tailed unpaired Student's test. A value of $p < 0.01$ was considered to be statistically significant.

Results

Expansion of the interventricular boundary region and absence of the IVS in Hand2 transgenic embryos

We first examined the expression pattern of *Hand2* during ventricular chamber and septum formation. At E9.5 and E10.5, *Hand2* expression was detected in the RV as well as in the LV and atria, but was absent in the interventricular boundary region (Figs. 1A and B). At E11.5, expression of *Hand2* was already down-regulated (Fig. 1C). The absence of *Hand2* expression in the boundary region suggested that like *Hand1*, *Hand2* may be also involved in IVS formation.

To investigate the effect of ventricular expression of *Hand2* on IVS and ventricular chamber formation, we generated transgenic mice expressing *Hand2* in the whole ventricles using the *β-MHC* promoter (Figs. 1D–F). Since our purpose was to determine a role of *Hand2* during cardiac development, we analyzed F0 transgenic embryos. Expression of Myc-tagged Hand2 protein and *Hand2* mRNA was confirmed by immunohistochemistry (Figs. 1E and F) and in situ hybridization (Fig. 1G), respectively.

At E10.5, the interventricular groove (IVG) was detected and the IVS at the apical side of the heart formed in wild type embryos (Figs. 1H and J), whereas no IVG or IVS formation could be observed in *Hand2* transgenic embryos (Figs. 1I and K). Moreover, in *Hand2* transgenic embryos, trabeculation occurred even in a region where the IVS was expected to form (Figs. 1I and K). *Bone morphogenetic protein 10* (*BMP10*) was used as a marker to distinguish trabecular from septal myocardium, since this gene is expressed in trabecular myocardium, but not in septal or compact zone myocardium [10]. At E11.5, although the IVS well formed in wild type hearts (Figs. 1L and N), only trabecular myocardium was observed in the inner lumen of the ventricle in *Hand2* transgenic embryos (Figs. 1M and O). Trabeculation was also observed in the interventricular boundary region in *Hand2* transgenic embryos (Figs. 1M and O), suggesting that *Hand2* may promote trabeculation.

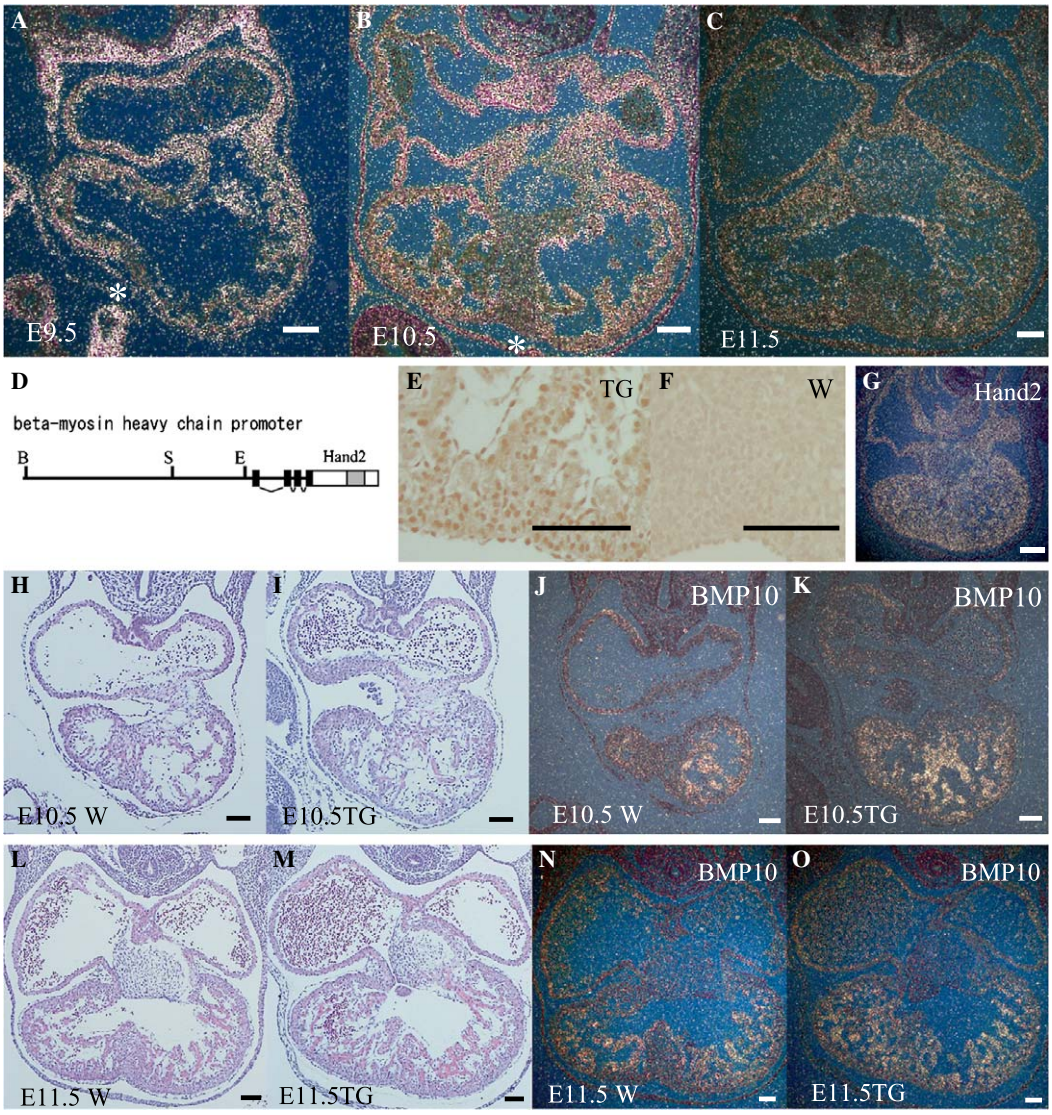

Fig. 1. (A–C) The expression pattern of *Hand2* during ventricular formation. Asterisks denote the interventricular boundary region. *Note*. Absence of *Hand2* expression in the boundary region. (D) Schematic representation of the transgene construct. Filled boxes represent four exons of the mouse β-myosin heavy chain (β-MHC) gene. The β-MHC promoter was ligated to Myc-tagged mouse *Hand2* cDNA with the human growth hormone poly(A) signal sequence. B: *Bam*HI, S: *Sal*I, E: *Eco*RI. (E,F) Immunohistochemistry using an anti-Myc antibody revealed expression of Myc-tagged Hand2 protein in ventricular myocytes of transgenic embryos (E), while no expression was observed in ventricular myocytes of wild type littermates (F). (G) In situ hybridization for the detection of *Hand2* showed homogeneous expression of *Hand2* in the ventricles including the boundary region in *Hand2* transgenic embryos. (H–O) Hematoxylin- and eosin-stained sections (H,I,L,M) and in situ hybridization for the detection of *BMP10* (J,K,N,O) are presented. Although, at E10.5, the IVS was observed in the apical part of the heart in wild type embryos (H,J), the IVS did not form and trabecular formation was detected even in the boundary region in *Hand2* transgenic embryos (I,K). At E11.5, the IVS formed in wild type hearts (L,N). *Note*. Trabeculation in the boundary region and absence of the IVS in *Hand2* transgenic hearts (M,O). Bars: 100 μm.

Some transgenic embryos showed heterogeneous expression of the transgene (Figs. 2A–D). In these transgenic embryos, the IVS well formed in the basal part of the heart where the *Hand2* transgene was not expressed in the boundary region (Figs. 2A and B), while the IVS formation was disturbed in the apical part of the heart where transgene expression of *Hand2* was detected in the boundary region (Figs. 2C and D), suggesting that IVS formation was tightly regulated by the expression domain of *Hand2*.

In *Hand2* transgenic hearts, *Hand1* expression was confined to the left side of the ventricle and the left-sided expression of *Tbx5* was not disturbed, indicating that there was a clear distinction between the left and right side of the ventricle at the molecular level, although there was no IVG or IVS (Figs. 2E–H). It was also shown that over-expression of *Hand2* did not affect the expression level of *Hand1* in contrast to the result that forced expression of *Hand1* suppressed *Hand2* expression [3]. A similar expression pattern and knock-out phenotype of *Versican*/*Cspg2* and *Hand2* suggested that *Versican* may be a downstream target gene of *Hand2* [2,11,12]. However, over-expression of *Hand2* in the whole ventricle did not disturb the right-dominant expression of *Versican* (Figs. 2I and J).

The above data indicated that *Hand2* expression caused expansion of the interventricular boundary region and that the absence of *Hand2* expression in this region may be critical for IVS formation. To confirm this result, we generated transgenic mice over-expressing a mutant form of *Hand2* as a control. It was reported that deletion of the helix–loop–helix (HLH) domain of *Hand2* (*Hand2-ΔHLH*) resulted in loss of both DNA binding and heterodimer forming activities, suggesting that *Hand2-ΔHLH* is unlikely to function as a dominant negative [13]. Moreover, transgenic expression of *Hand2-ΔHLH* in the forelimb bud did not cause any limb patterning defects [13], indicating that *Hand2-ΔHLH* could serve as a control. As expected, over-expression of *Hand2* without the HLH domain did not affect IVS or chamber formation (Figs. 3A–E).

Furthermore, we also considered a possibility that over-expression of *Hand2* in the ventricular walls might have affected the phenotype. To address this concern, we generated transgenic embryos over-expressing *Hand2* under the control of the rat *myosin heavy chain 2V* (*MLC2V*) promoter, driving transgene expression in the RV and outflow tract, but not in the boundary region. In these transgenic embryos, the IVS formed normally despite over-expression of *Hand2* in the RV wall, suggesting that the absence of *Hand2* expression in the boundary region was critical in the normal formation of the IVS (Figs. 3F–J).

Expression of Hand2 up-regulated ANF, BNP, and Connexin40 in vitro and in vivo

In *Hand2* transgenic embryos, trabeculation was observed even in a region where the IVS was expected to form. To test if *Hand2* expression promotes differentiation of cardiac myocytes into trabecular myocardium, we transfected H9c2 cells, an embryonic myocardial cell line, with *Hand2* cDNA. Expression of *Hand2* in H9c2 cells significantly up-regulated expression of *ANF*, *BNP*, and *Connexin40* (*Cx40*), all of which are known to be up-regulated in trabecular myocardium [14–16], while expression of *β-myosin heavy chain*, expressed both in trabecular and compact layer myocardium, was comparable between cells transfected with *Hand2* and *Hand2*-ΔHLH (Figs. 4A–D). We also investigated the effects of *Hand2* on expression of these genes in vivo. Up-regulation of *ANF*, *BNP*, and *Cx40* was observed in transgenic embryos over-expressing *Hand2*, but not in transgenic embryos over-expressing *Hand2*-ΔHLH (Figs. 4E–M).

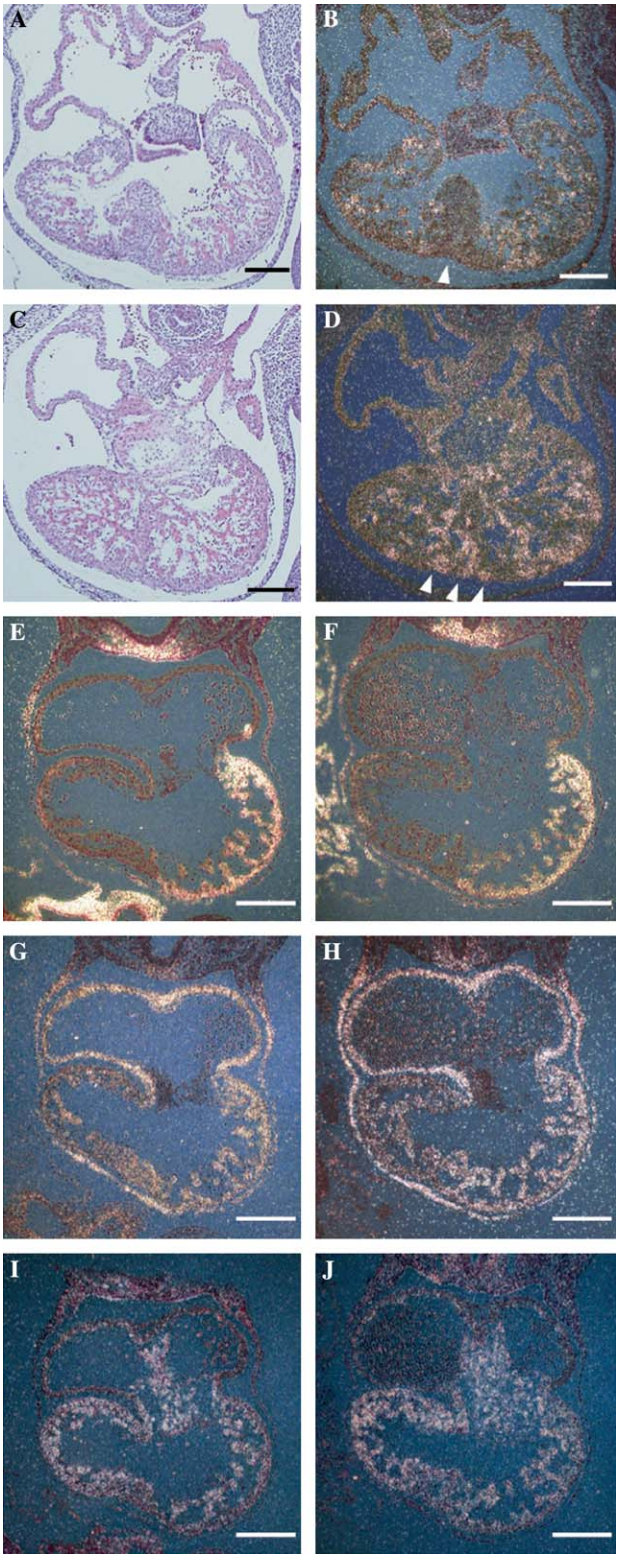

Fig. 2. (A–D) In embryos exhibiting heterogeneous expression of the transgene, the IVS formed in the basal part of the heart where *Hand2* was not expressed in the boundary region (A,B). In contrast, in the apical part of the heart where *Hand2* expression was observed in the boundary region (arrowheads in D), IVS formation was disturbed (C,D). (E–H) Expression of *Hand1* (E,F), *Tbx5* (G,H), and *versican*/*Cspg2* (I,J) in wild type (E,G, and I) and *Hand2* transgenic (F,H, and J) embryos is shown. The left-dominant expression of *Hand1* and *Tbx5* was not disturbed, indicating a clear distinction between the RV and LV in transgenic hearts (F,H). It was also shown that forced expression of *Hand2* did not affect *Hand1* expression (F). *Versican*/*Cspg2* was supposed to be a downstream gene of *Hand2*, but the RV-dominant expression pattern was conserved in transgenic embryos over-expressing *Hand2* in the LV (I,J).

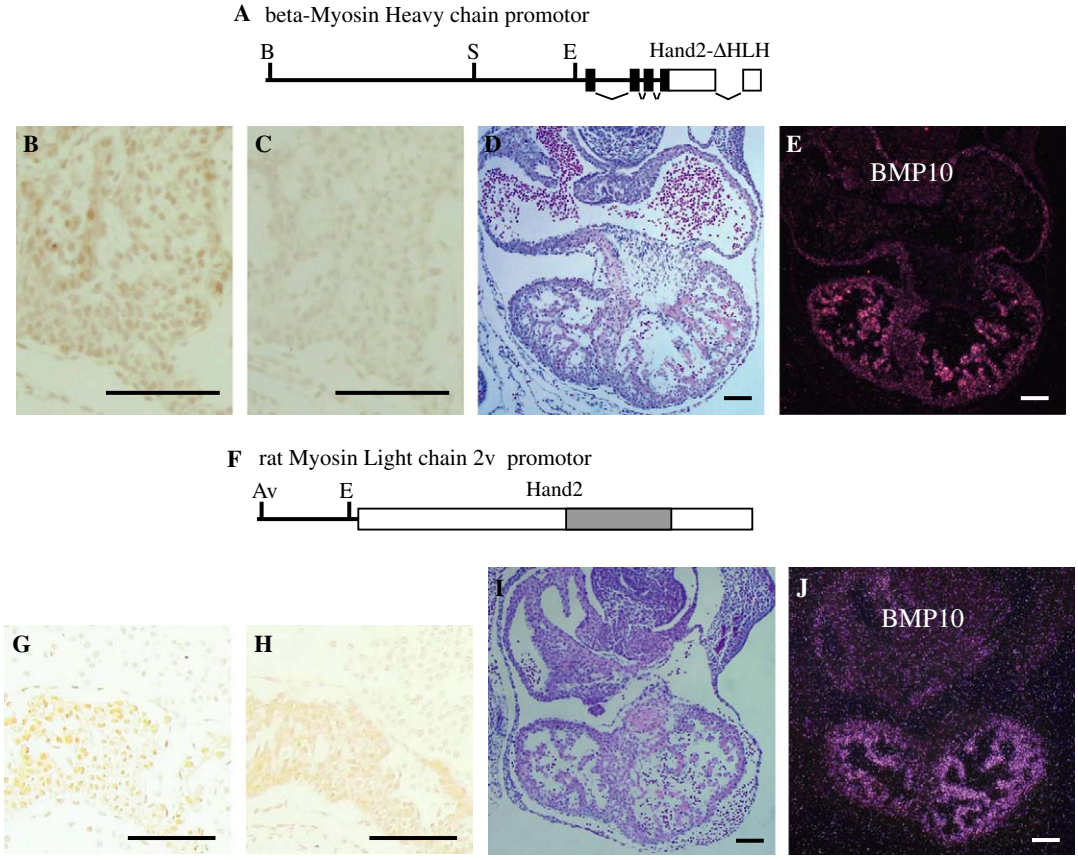

Fig. 3. (A) Schematic representation of the transgene construct. The construct was designed for expressing a mutant form of *Hand2*, lacking the helix–loop–helix domain, in the whole ventricle. B: *Bam*HI, S: *Sal*I, and E: *Eco*RI. (B,C) Expression of the mutant form of Hand2 protein in ventricular myocytes of transgenic embryos was confirmed by immunohistochemistry using an anti-FLAG antibody (B). No expression was detected in ventricular myocytes of wild type littermates (C). (D,E) The IVS formed normally in the transgenic embryos expressing the mutant form of *Hand2* in the whole ventricle. (F) Schematic representation of the transgene construct. The rat myosin light chain 2V promoter was ligated to Myc-tagged mouse *Hand2* cDNA with the human growth hormone poly(A) signal sequence. Av: *Ava*II, E: *Eco*RI. (G,H) Expression of the Hand2 protein in ventricular myocytes of transgenic embryos was confirmed by immunohistochemistry using an anti-Myc antibody (G). No expression was detected in ventricular myocytes of wild type littermates (H). (I,J) The IVS formed normally in the transgenic embryos expressing *Hand2* under the control of the rat myosin light chain 2V promoter. Expression of *BMP10*, a molecular marker of trabecular myocardium, was examined to distinguish the IVS from the trabeculae (E,J). Bars: 100 μm.

Discussion

The present study demonstrated that the absence of *Hand2* expression in the boundary region was critical for IVS formation. Together with the previous work [3], we concluded that absence of both *Hand1* and *Hand2* expression in the boundary region was required for IVS formation. What is the significance of lack of *Hand1* and *Hand2* expression in the boundary region? Lack of the *Hand* genes in this region may be critical for two major steps of IVS formation: initial formation and subsequent growth of a septum. First, a region where a septum is expected to form should not expand for initial formation of a septum [17]. Since both *Hand1* and *Hand2* are involved in expansion of ventricular walls, it is reasonable that absence of the *Hand* genes in the boundary region was essential for IVS formation. Second, maintenance of proliferative capacity in septal myocardium is required for growth of a septum. In mammals, septal myocardium arises from compact zone myocardium [18] and has higher proliferative capacity than trabecular myocardium [19]. Thus, inhibition of differentiation into trabecular myocardium in the boundary region may be important for growth of a septum. Since trabecular formation was observed even in the interventricular boundary region in *Hand2* transgenic embryos and *Hand2* induced expression of molecular markers for trabecular myocardium both in vitro and vivo, it is possible that *Hand2* may regulate trabeculation and that lack of expression of *Hand2* may inhibit trabecular formation in the boundary region. This is consistent with the phenotype of homozygous mutant mice for *Hand2*, exhibiting absence of trabecular formation [2]. Interestingly, a retrospective clonal approach revealed that cardiac myocytes in the interventricular region originated from a subpopulation of cardiac myocytes distinct from left and right ventricular myocytes [20]. These cells were negative for *Hand1* and *ANF* [3,14], and the present study showed that the cells were also negative for *Hand2*. Forced expression of *Hand2* in these cells may change their specific characteristics, resulting in trabeculation and a lack of septal

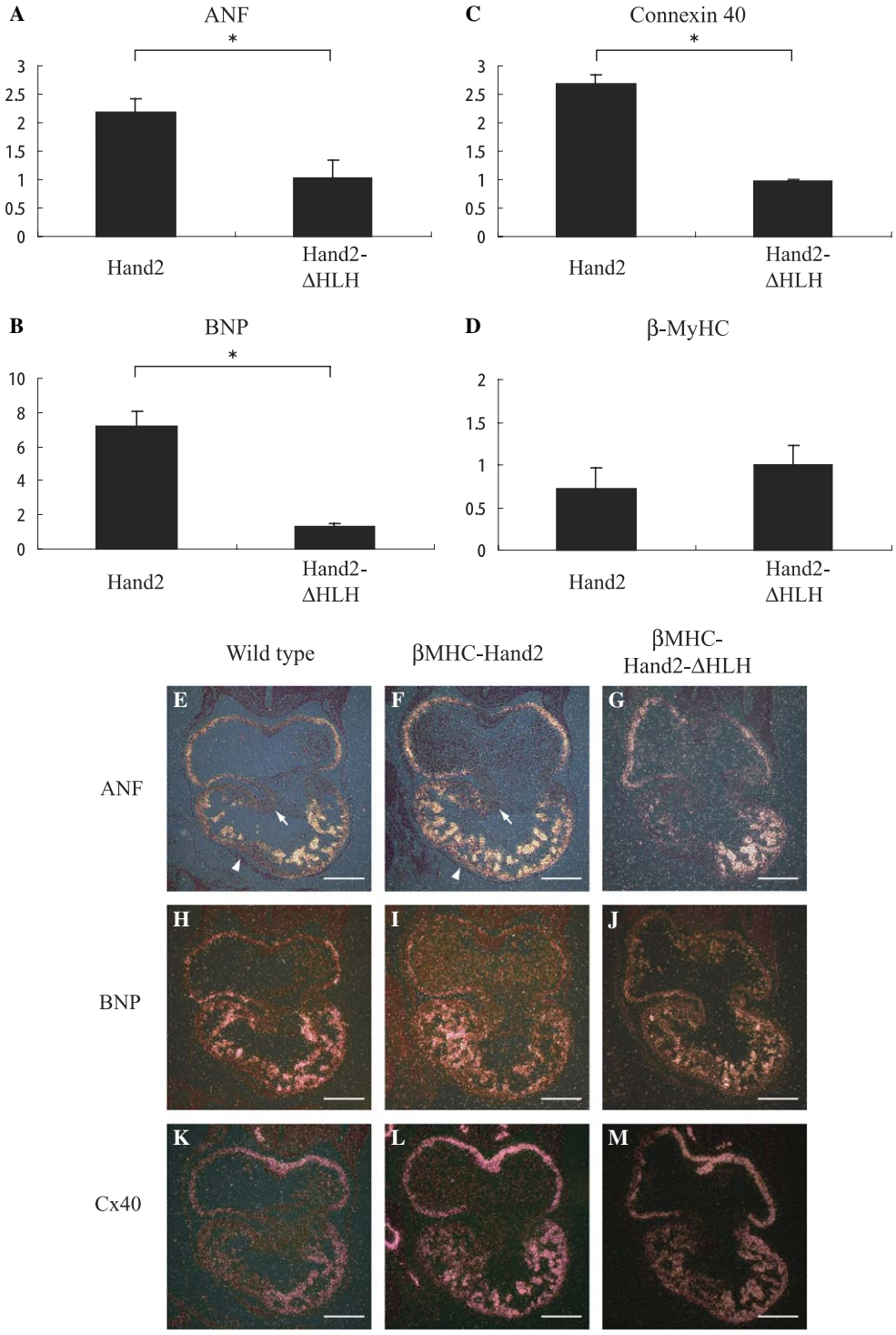

Fig. 4. (A–D) Quantitative RT-PCR analyses of *ANF* (A), *BNP* (B), *connexin40* (C), and *β-myosin heavy chain* (D) are presented. Expression of *ANF*, *BNP*, and *connexin40* (*Cx40*), predominantly expressed in trabecular myocardium, was up-regulated in H9c2 cells transfected with *Hand2* ($*P < 0.01$), while expression of *Hand2-ΔHLH* showed no effects. In contrast, expression of *β-myosin heavy chain*, expressed both in trabecular and compact layer myocardium, was comparable between cells transfected with *Hand2* and *Hand2-ΔHLH*. *Y*-axes represent the fold of activation of gene expression normalized to data obtained from vector-only transfected cells. (E–M) Effect of *Hand2* expression on gene expression patterns of *ANF*, *BNP*, and *Cx40* in vivo. Expression of *ANF* (E–G), *BNP* (H–J) and *Cx40* (K–M) in wild type (E,H, and K), *β-MHC-Hand2* transgenic (F,I, and L) and *β-MHC-Hand2-ΔHLH* transgenic (G,J, and M) embryos are shown. In *β-MHC-Hand2* transgenic embryos, *ANF* expression was up-regulated in the RV and ectopic expression of *ANF* was observed in the boundary region. Up-regulation of *ANF* was not observed in *β-MHC-Hand2-ΔHLH* transgenic embryos (G). Expression of *BNP* and *Cx40* was also up-regulated in the RV and boundary region of *β-MHC-Hand2* transgenic ventricle (I,L), but not in those of *β-MHC-Hand2-ΔHLH* transgenic (J,M). Bars: 100 μm.

growth in the interventricular boundary region. Furthermore, expansion of the outer curvature of the ventricle occurs independently of trabecular formation, because the IVS and ventricular chambers formed normally in the heart of knock-out mice homozygous for ErbB2 or ErbB4 that lack trabeculation [21,22]. Therefore, *Hand2* may regulate expansion and trabeculation through separate molecular mechanisms.

The transgenic data could be interpreted to mean that the IVS forms in an area making the border between *Hand1* and *Hand2* expression. We generated and analyzed transgenic embryos over-expressing *Hand1* under the control of a 3.3 kb upstream regulatory sequence of *Nkx2.5* that drives transgene expression in the distal part of the RV and outflow tract [23]. Transgene expression of *Hand1* in the distal part of the RV created another border between *Hand1* and *Hand2* expression in the RV. However, we observed no additional septum formation in the RV of the transgenic embryos (K.T. and M.T. unpublished data). Therefore, it may be unlikely that the IVS forms in an area marking the border between *Hand1* and *Hand2* expression.

Up-regulation of *ANF*, *BNP*, and *Cx40* was observed in the RV and the boundary region of *Hand2* transgenic mice, but significant up-regulation of these genes was not detected in the LV. In our previous report, we demonstrated that *Hand1*, strongly expressed in the LV, also regulated *ANF* expression [3]. Although it is unknown whether *Hand1* regulates expression of *BNP* or *Cx40*, the dominant expression of *Hand1* in the LV may be the reason why exogenous *Hand2* was less effective in the LV than in the RV and boundary region.

Zebrafish has a single *Hand* gene, which is closely related to the mammalian *Hand2* gene [24]. The zebrafish *Hand* gene is homogeneously expressed in the ventricle, resulting in concentric expansion of a single ventricle. During evolution, a second *Hand* gene, *Hand1*, was added to the left half of the ventricle, dividing the ventricular chamber into two parts: one with *Hand1* expression and one without. At the same time, a subpopulation of cardiac myocytes negative for the *Hand* genes appeared at the midline of the ventricle, leading to formation of the boundary region. Molecular mechanisms determining the expression domains of *Hand1* and *Hand2* in the embryonic heart await further investigation.

In summary, ventricular expression of *Hand2* disrupted IVS formation. The absence of *Hand2* expression and lack of trabeculation in the interventricular boundary region may be critical for IVS formation. Left-sided expression of *Hand1* and absence of both *Hand1* and *Hand2* expression in the midline of the ventricle may have played an important role in the creation of mammalian hearts with characteristic right and left ventricles and a complete interventricular septum.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2006.02.122.

References

[1] C.G. Farmer, Evolution of the vertebrate cardio-pulmonary system, Annu. Rev. Physiol. 61 (1999) 573–592.
[2] D. Srivastava, T. Thomas, Q. Lin, M.L. Kirby, D. Brown, E.N. Olson, Regulation of cardiac mesodermal and neural crest development by the bHLH transcription factor, dHAND, Nat. Genet. 16 (1997) 154–160.
[3] K. Togi, T. Kawamoto, R. Yamauchi, Y. Yoshida, T. Kita, M. Tanaka, Role of Hand1/eHAND in the dorso-ventral patterning and interventricular septum formation in the embryonic heart, Mol. Cell. Biol. 24 (2004) 4627–4635.
[4] D.G. McFadden, A.C. Barbosa, J.A. Richardson, M.D. Schneider, D. Srivastava, E.N. Olson, The Hand1 and Hand2 transcription factors regulate expansion of the embryonic cardiac ventricles in a gene dosage-dependent manner, Development 132 (2005) 189–201.
[5] H. Rindt, S. Knotts, J. Robbins, Segregation of cardiac and skeletal muscle-specific regulatory elements of the beta-myosin heavy chain gene, Proc. Natl. Acad. Sci. USA 92 (1995) 1540–1544.
[6] S.A. Henderson, M. Spencer, A. Sen, C. Kumar, M.A. Siddiqui, K.R. Chien, Structure, organization, and expression of the rat cardiac myosin light chain-2 gene. Identification of a 250-base pair fragment which confers cardiac-specific expression, J. Biol. Chem. 264 (1989) 18142–18148.
[7] B. Hogan, R. Beddington, F. Constantini, E. Lacy, Manipulating the Mouse Embryo, Cold Spring Harbor Laboratory Press, New York, 1994.
[8] M. Tanaka, Z. Chen, S. Bartunkova, N. Yamasaki, S. Izumo, The cardiac homeobox gene Csx/Nkx2.5 lies genetically upstream of multiple genes essential for heart development, Development 126 (1999) 1269–1280.
[9] Y. Takebe, M. Seiki, J. Fujisawa, P. Hoy, K. Yokota, K. Arai, M. Yoshida, N. Arai, SR alpha promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the R-U5 segment of human T-cell leukemia virus type 1 long terminal repeat, Mol. Cell. Biol. 8 (1988) 466–472.
[10] H. Neuhaus, V. Rosen, R.S. Thies, Heart specific expression of mouse BMP-10 a novel member of the TGF-beta superfamily, Mech. Dev. 80 (1999) 181–184.
[11] D.J. Henderson, A.J. Copp, Versican expression is associated with chamber specification, septation, and valvulogenesis in the developing mouse heart, Circ. Res. 83 (1998) 523–532.
[12] C.H. Mjaatvedt, H. Yamamura, A.A. Capehart, D. Turner, R.R. Markwald, The Cspg2 gene, disrupted in the hdf mutant, is required for right cardiac chamber and endocardial cushion formation, Dev. Biol. 202 (1998) 56–66.
[13] D.G. McFadden, J. McAnally, J.A. Richardson, J. Charite, E.N. Olson, Misexpression of dHAND induces ectopic digits in the developing limb bud in the absence of direct DNA binding, Development 129 (2002) 3077–3088.

[14] V.M. Christoffels, P.E. Habets, D. Franco, M. Campione, F. de Jong, W.H. Lamers, Z.Z. Bao, S. Palmer, C. Biben, R.P. Harvey, A.F. Moorman, Chamber formation and morphogenesis in the developing mammalian heart, Dev. Biol. 223 (2000) 266–278.
[15] V.A. Cameron, G.D. Aitken, L.J. Ellmers, M.A. Kennedy, E.A. Espiner, The sites of gene expression of atrial, brain, and C-type natriuretic peptides in mouse fetal development: temporal changes in embryos and placenta, Endocrinology 137 (1996) 817–824.
[16] S.R. Coppen, R.A. Kaba, D. Halliday, E. Dupont, J.N. Skepper, S. Elneil, N.J. Severs, Comparison of connexin expression patterns in the developing mouse heart and human foetal heart, Mol. Cell. Biochem. 242 (2003) 121–127.
[17] T.W. Sadler, Langman's Medical Embryology, ninth edition., Lippincott William & Wilkins, Baltimore, 2004.
[18] D. Sedmera, T. Pexieder, M. Vuillemin, R.P. Thompson, R.H. Anderson, Developmental patterning of the myocardium, Anat. Rec. 258 (2000) 319–337.
[19] D. Sedmera, M. Reckova, A. DeAlmeida, S.R. Coppen, S.W. Kubalak, R.G. Gourdie, R.P. Thompson, Spatiotemporal pattern of commitment to slowed proliferation in the embryonic mouse heart indicates progressive differentiation of the cardiac conduction system, Anat. Rec. A Discov. Mol. Cell. Evol. Biol. 274 (2003) 773–777.
[20] S.M. Meilhac, M. Esner, M. Kerszberg, J.E. Moss, M.E. Buckingham, Oriented clonal cell growth in the developing mouse myocardium underlies cardiac morphogenesis, J. Cell Biol. 164 (2004) 97–109.
[21] K.F. Lee, H. Simon, H. Chen, B. Bates, M.C. Hung, C. Hauser, Requirement for neuregulin receptor erbB2 in neural and cardiac development, Nature 378 (1995) 394–398.
[22] M. Gassmann, F. Casagranda, D. Orioli, H. Simon, C. Lai, R. Klein, G. Lemke, Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor, Nature 378 (1995) 390–394.
[23] M. Tanaka, S.B. Wechsler, I.W. Lee, N. Yamasaki, J.A. Lawitts, S. Izumo, Complex modular *cis*-acting elements regulate expression of the cardiac specifying homeobox gene Csx/Nkx2.5, Development 126 (1999) 1439–1450.
[24] D. Yelon, B. Ticho, M.E. Halpern, I. Ruvinsky, R.K. Ho, L.M. Silver, D.Y. Stainier, The bHLH transcription factor hand2 plays parallel roles in zebrafish heart and pectoral fin development, Development 127 (2000) 2573–2582.