Expression of Type XVII Collagen $\alpha 1$ Chain mRNA in the Mouse Heart

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SUMMARY

The type XVII collagen $\alpha 1$ chain has been identified as a component of the type I hemidesmosome, and is thus thought to play a role in extracellular matrix (ECM) maintenance and signal transduction between the cell and the ECM. We examined the expression of type XVII collagen $\alpha 1$ chain mRNA in the mouse heart by Northern blot analysis and determined the sequential changes of its expression in different developmental stages of the heart using the reverse transcriptase-polymerase chain reaction (RT-PCR) method. *Northern blotting:* Total RNA was extracted from 10 adult mouse hearts by the guanidine/cesium method. Hybridization was performed with mouse cDNA for $\alpha 1$ (XVII) collagen. *RT-PCR:* Total RNA was extracted from 7 embryos , 4 neonates and 8 adult mice. Reverse transcription was performed using oligo-dT primer and MMLV. Amplification was carried out in $\alpha 1$ (XVII) collagen and glyceraldehyde 3-phosphate dehydrogenase (GAPDH). GAPDH served as an internal control. Northern blotting revealed a 5.6 kb signal that was identical to that of the $\alpha 1$ (XVII) of skin and transformed keratinocyte reported previously. The sequences of the PCR products were also identical to those reported. The normalized expression ratios of $\alpha 1$ (XVII) were 0.91 ± 0.20 in the embryonic heart, 0.36 ± 0.20 in the neonatal heart and 0.96 ± 0.21 in the adult heart. In conclusion, we identified the expression of type XVII collagen $\alpha 1$ chain mRNA in the mouse heart, suggesting that the type I hemidesmosome is located in the heart. The results of the RT-PCR at different developmental stages of the heart suggest that type XVII collagen contributes not only to cardiogenesis in the embryonic stage but also to maintenance of architecture and function in the adult heart. (Jpn Heart J 1998; 39: 211–220)

Key words: Molecular biology, Extracellular matrix, Basement membrane, Reverse transcriptase-polymerase chain reaction, Myocardium

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HEMIDESMOSOMES are unique adhesion complexes that mediate cell adhesion to the extracellular matrix (ECM) component through the association of the filament into cytoplasmic plaques.[1-4]

The molecular aspects of hemidesmosomes have recently been clarified.[5] Two types of hemidesmosome have been identified: types I and II.[6-8] The type I hemidesmosome, which is the typical hemidesmosome, is composed of 5 major distinct polypeptides with molecular masses of 500, 230, 200, 180 and 120 kDa, sequentially termed hemidesmosome 1 to hemidesmosome 5 (HD1-HD5).[6-15] The type II hemidesmosome consists of HD1, HD3 and HD5 molecules but lacks HD2 and HD4. The type II hemidesmosome is found in certain endothelial cells and glial cells.[8]

HD4 is a 180 kDa bullous pemphigoid antigen (formerly designated as BP180) that was initially isolated as an autoantigen in autoimmune subepidermal blistering disease, bullous pemphigoid.[16,17] The HD4 (BP180) molecule is a transmembrane protein with a C-terminal extracellular segment consisting of 15 interrupted collagenous domains, and has been recently designated as type XVII collagen.[18] Li *et al.*[18] recently cloned the type XVII $\alpha 1$ chain.

Although the biological role of hemidesmosomes has not been fully clarified, based on the characteristic structure of the hemidesmosome, it has been suggested that the hemidesmosome plays a role in ECM maintenance and signal transduction between the cells and the ECM.

We hypothesized that type XVII collagen is expressed in the heart. Although the distribution of the expression of HD4 (BP180) in other tissues has been reported,[19] there have been no reports regarding the expression of type XVII collagen in the heart. Accordingly, we examined the expression of type XVII collagen $\alpha 1$ chain mRNA in mouse hearts by Northern blot analysis, and identified the sequential changes of its expression in different developmental stages of the heart using the reverse transcriptase-polymerase chain reaction (RT-PCR) method.

Materials and Methods

Materials

A total of 10 BALb/c mice (Okayama University Medical School Animal Center, Okayama, Japan) were used to determine the expression of type XVII $\alpha 1$ [$\alpha 1$(XVII)] chain mRNA expression by Northern blotting. To examine the expression at different stages of mouse heart development by RT-PCR, 7 mice at the embryonic stage (day-18 to -20), 4 neonatal (1-day old), and 8 adults (2-months old) hearts were used.

Methods

Northern blotting

Isolation of RNA. Total RNA was extracted from the hearts by the guanidium isothiocyanate-cesium chloride density gradient centrifugation method reported previously.[20,21] The recovery of RNA was calculated from the optical densities at 260 nm and the purity of extracted RNA confirmed from the optical density ratio of 260 nm/280 nm. A 1 μg mRNA, which was purified from 100 μg total RNA extracted from the heart, and 30 μg each of total RNA extracted from tongue and from PAM cells were electrophoresed on 0.8% agarose-formaldehyde gels containing 2.2M formaldehyde. They were then transferred to Hybond N nylon membranes (Amersham, Arlington, Heights, IL, USA)[21] and then to Hybond N nylon membranes (Amersham).[22] After ultraviolet cross-linking, filters were prehybridized in a solution containing 50% formamide, 6 × SSC (1 × SSC; 300 nM/L sodium chloride and 30 nM/*l* sodium citrate, pH 7.0), Denhardt's solution (i.e., 0.1% bovine serum albumin, 0.1% Ficoll 400 and 0.1% polyvinylpyrrolidone), 0.5% sodium dodecyl sulfate (SDS) and 20 μg/m*l* of heated denatured salmon sperm deoxyribonucleic acid at 55°C for 3 hours.

Hybridization. Recombinant plasmids complementary to mouse α1 (XVII) containing 370 base pairs (bp), which were prepared according to the methods previously reported,[23] were used for making cDNA probe. The electrophoresed mRNAs on filters were hybridized with a ^{32}P-labeled mouse α1 (XVII) complementary deoxyribonucleic acid (cDNA) probe in the same solution as that used for prehybridization at 55°C for 24 hours. The cDNAs were labeled with ^{32}P dCTP by the random primer extension method reported by Feinberg and Volgelstein,[23] and Church and Gilbert[24] using the Amersham Multiprime DNA labeling kit. After hybridization, the filters were washed under stringent conditions: once with 3 × SCC containing 0.1% SDS at 55°C for 30 min, once with 2 × SSC containing 0.1% SDS at 55°C for 30 min, and once with 0.1 × SSC containing 0.1% SDS at room temperature for 15 min. Radio-labeled filters were exposed to x-ray film (Kodak XAR, Eastman Kodak Co., Rochester, NY, USA) at −70°C with an intensifying screen.

RT-PCR

Isolation of RNA. We employed RT-PCR to examine the relative expression of the α1(XVII) chain mRNA in the hearts examined, because a relatively small amout of RNA could be extracted from the embryonic hearts. Total RNA was extracted from the mouse heart at different developmental stages using a RNAZol™ kit (Cinna Biotecx Lab., Houston, TX, USA) according to the established method.[25] Approximately >30 μg RNA was obtained from the heart at each stage (embryonic, neonatal, and adult). First strand cDNA was synthesized using 2 μg of total RNA extracted from each stage and used for the template/

reaction in 20 μl of a solution containing 2.5 mM dNTPs (Pharmacia Biotech, Tokyo, Japan), 125 units of Moloney murine leukemia virus reverse transcriptase (USBTM, Cleveland, OH, USA), 20 units of RNasin (Toyobo, Osaka, Japan) and 100 pmol of Oligo $(dT)_{15}$ primer (Promega, Madison, WI, USA). A 2.5 μl aliquot of cDNA material with Taq DNA polymerase (Toyobo) was used to amplify the α1(XVII) chain mRNA by PCR. The primers specific for α1(XVII) mRNA and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) were designed by Oligo 4.0 (National Biosciences Inc., Plymouth, MN, USA) and synthesized by a Model 394 DNA Synthesizer (Perkin Elmer Japan, Urayasu, Chiba, Japan).

Number of PCR Cycles. Initially, PCR was performed with 10 cycles and the number of cycles was then increased by 2 cycles. The densities of the amount of PCR products were plotted against the number of cycles. Finally, the number of cycles was determined as the number that showed a linear trajectory before it reached the maximum plateau. PCR determinations for all samples were performed at the same time using a Thermal Cycler™ (MJ Research Inc., Watertown, MA, USA).

Condition for PCR. The specific primers and the distance between the forward and reverse primers were as follows.

α1(XVII): (94°C – 1 min, 60°C – 2 min, 70°C – 3 min) × 30 cycle, 70°C – 7 min, 4°C –.

GAPDH: (94°C – 1 min, 52°C – 2 min, 70°C – 3 min) × 24 cycle, 70°C – 7 min, 4°C –.

forward direction of the primer:

α1(XVII): 5′-ATGACTTCAGAGGGATCATCAACAATCACT-3′

GAPDH(mouse): 5′-GCCAAACGGGTCATCATCTC-3′

reverse direction

α1(XVII): 5′-AGTGAAGAAGGTTTCTGAGTCAGTCAG-3′

GAPDH(mouse): 5′-CACATTGGGGGTAGGAACAC-3′

distance between forward and reverse primers:

α1(XVII); 370 bp, GAPDH; 375 bp.

The PCR fragments were visualized by 1% agarose gel electrophoresis and ethidium bromide staining.

Southern blotting. The electrophoresed products on 1% agarose gels were also blotted and fixed onto nylon filters (Hybond™-N+; Amersham, Little Chalfout, Buckinghamshire, UK) with 0.4 N NaOH and hybridized with the complementary DNA probe. Probes were labelled routinely to a specific activity of 1×10^6

cpm/m*l* of DNA using a random prime kit (Promega).
Sequencing of PCR Product. RT-PCR product from total RNA of PAM cells was ligated into the pCR™ 2.1 vector using TA cloning kit (Invitrogen Corp., San Diego, CA, USA). Nucleotide sequence analysis was perfomed by the dideoxy-chain termination technique. The fluorescence labeled dye-terminator method and an automatic sequencer (Model 373S, Perkin Elmer Japan) were used.
Quantification. The bands of electrophoresed amplified products hybridized for amplified α1(XVII) mRNA were quantified densitometrically using a Fast Scan Densitometer (Molecular Dynamics Co., Sunnyvale, CA, USA) and normalized relative to the GAPDH band density.

Statistical analysis

To compare the expression of α1(XVII) chain mRNA in the embryonic, neonatal and adult hearts, analysis of variance with Sheffe's F-test was employed for the comparison of group data. All values are expressed as mean ± SD, and a p value <0.05 was accepted as statistically significant.

Results

Northern blotting

The RNA extracted from the adult hearts was positively hybridized with α1(XVII) cDNA by Northern blotting. The exposed band is shown in Figure 1. The hybridized signals were positioned at 5.6Kb with a single band. The signals were identical to those in the controls (PAM cell and tongue).

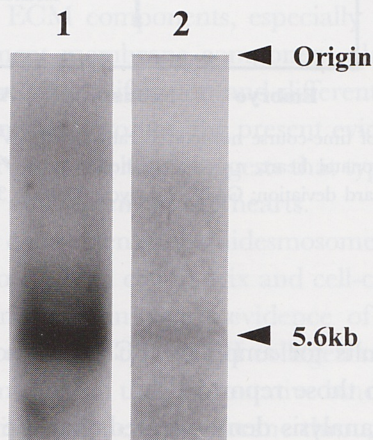

Figure 1. Bands expressed by Northern blotting. **Lane 1.** PAM cells RNA (positve control); **Lane 2.** Adult mouse heart RNA. A clear single band can be seen at 5.6 Kb.

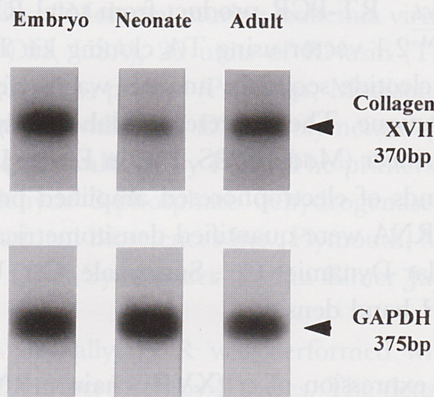

Figure 2. Results of Southern blotting for amplified α1(XVII) mRNA by PCR. The PCR products are positively hybridized with α1 (XVII) collagen cDNA. The intensity of the band in the neonatal heart is weaker than that in embryonic or adult heart.

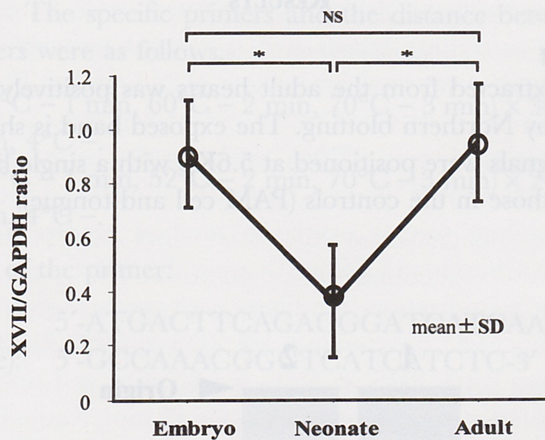

Figure 3. Results of time-course normalized ratio of α1(XVII)/GAPDH. embryonic heart, $n=7$; neonatal heart, $n=4$; adult heart, $n=8$. *significant difference ($p<0.05$); SD = standard deviation; GAPDH = glyceraldehyde 3-phosphate dehydrogenase.

RT-PCR

The sequential results for amplified PCR products of α1(XVII) were in complete agreement with those reported.[18]

The southern blot analysis demonstrated that the band amplified by RT-PCR hybridized with α1(XVII) cDNA. (Figure 2). The bands were positioned at 370 bp with a single band. The southern blot analysis of amplified α1(XVII) by

PCR demonstrated that α1(XVII) collagen mRNA was expressed in all of the stages of the heart examined, i.e., the embryonic, neonatal and adult hearts (Figure 2). The normalized expression ratios of α1(XVII) to GAPDH were 0.91 ± 0.20 ($n = 7$) in the embryonic heart, 0.36 ± 0.20 ($n = 4$) in the neonatal heart and 0.96 ± 0.21 ($n = 8$) in the adult heart (Figure 3). The expression of α1(XVII) chain mRNA in the neonatal hearts was significantly lower than that in the embryonic or adult hearts. No differences in its expression between the embryonic and adult hearts were observed.

Discussion

The findings of present study first have demonstrated the expression of type XVII collagen α1 chain mRNA in the heart of mice. RT-PCR revealed that it was expressed through the embryonic to adult-heart stages. These findings suggest that type XVII collagen contributes not only to cardiogenesis in the embryonic stage but also to maintenance of architecture and function in the adult heart.

HD4 (BP180) has 3 structural domains; an intercellular domain, a cell-membrane spanning domain and an extracellular collagenous domain.[5] The amino acid sequence of HD4 meets the criteria of collagenous protein and was designated as type XVII collagen.[18] The type XVII collagen α1 chain contains of 13 distinct collagenous domains with noncollagenous interruptions. The high interrupted triple helices are similar to those in types IV and VII collagen. The amino-terminal of type XVII collagen is connected to a cell-membrane spanning noncollagenous domain. Based on these characteristics of type XVII collagen and its location in the skin, type XVII collagen is thought to serve as an attachment site for the other ECM components, especially the basement membrane components. The basement membrane component plays the important role of providing cue signals for cell proliferation and differentiation.[26] In light of these biological findings in hemidesmosomes, the present evidence of the expression of type XVII collagen mRNA in the heart suggests that type XVII collagen contributes to the cell-to-ECM attachment in the hearts.

Integrin is another component of hemidesmosome.[27] Integrins act as receptors for ECM proteins to mediate cell-matrix and cell-cell interactions. Although the present study did not obtain direct evidence of an association between integrin and type XVII collagen, type XVII collagen may contribute to cell-cell and cell-ECM signal transduction through putative binding sites with ECM components, in particular with basement membrane through integrins.

We demonstrated type XVII collagen expression not only in the adult heart but also in the embryonic heart. Although hemidesmosomes in skin have been

studied extensively, there have been no reports on the expression of hemidesmosome components in different developmental stages of other organs. The close relation of type XVII collagen and basement membrane indicates as mentioned above that type XVII collagen contributes to the matrix-cell interaction to maintain the cardiac architecture and function. A close relation of a hemidesmosomal component with laminin through integrin has been revealed.[27] Developmental studies[28] have demonstrated that laminin as well as other basement membrane components plays an essential role in embryogenesis. The present findings of the expression of type XVII collagen indicate that it contributes to the embryogenesis of the heart, through an interaction with matrix receptor and basement membrane components.

Five component molecules of hemidesmosome have been identified. The present study elucidated type XVII $\alpha 1$ chain mRNA expression, indicating that hemidesmosomes in the heart are type I. We did not, however, examine the expression of other components associated with type XVII collagen. Because of the complexity of hemidesmosomal components, the possibility that the hemidesmosome including the type XVII collagen in the heart might be different from the skin hemidesmosome, i.e., another type of hemidesmosome, cannot be excluded. We cannot make any further conclusion regarding the components of hemidesmosomes in which type XVII collagen resides in the heart, though type I hemidesmosome is the most likely.

The present study revealed that the expression of $\alpha 1$(XVII) collagen mRNA in neonatal hearts was lower than that in the embryonic or adult hearts. The hemodynamic activity in the neonatal hearts is different from that in the adult heart, and this might partly account for the low $\alpha 1$(XVII) collagen mRNA expression compared to the adult hearts.

This study did not clarify the role of type XVII collagen in response to hemodynamic changes in the hearts. One study[29] demonstrated that pressure overload increases basement membrane collagen (type IV collagen). As stated above, the close association of type XVII collagen with the basement membrane has been reported. Type IV collagen might thus play some role in adaptation of ECM and/or myocytes in response to hemodynamic load through its contribution to cell-to-ECM attachment, and cell-cell and cell-ECM signal transduction.

In summary, the present study has demonstrated the expression of type XVII $\alpha 1$ chain mRNA in embryonic, neonatal and adult mouse hearts, suggesting its contribution to cardiogenesis and to the maintenance of heart architecture and function.

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