

심장판막에서 TGF- β 1의 생성과 분포고재기¹ · 김남균² · 김민호¹ · 채제건¹ · 고규영^{1,3}The Synthesis and Distribution of TGF- β 1 in Cardiac ValvesJae-Ki Ko, MD¹, Nam-Gyun Kim, PhD², Min Ho Kim, MD¹,
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ABSTRACT

Background and Objectives : Transforming growth factor- β 1 (TGF- β 1) plays an important role on cardiac muscle differentiation, cardiac septa and valve formation during heart development. However, the role of TGF- β 1 in cardiac valves of adult animals is largely unknown. Cardiac valves are target portion from repetitive, periodic and continuous physical loading in the body. Therefore, we examined the mRNA, protein levels, and protein distribution of TGF- β 1 in cardiac valves of adult animals to clarify the biological importance of TGF- β 1. **Materials and Methods :** Adult mice, rats and pigs were used. Cardiac valves of pig were frozen and were pulverized with liquid nitrogen. To measure the mRNA levels of TGF- β 1 in cardiac valves, total RNA was extracted using Tri-reagent and performed Northern blot analysis. To measure the protein levels of TGF- β 1 in cardiac valves, total protein was extracted and performed Western blot analysis. To examine the TGF- β 1 distribution, immuno-histochemistry with anti-CC-1-30 antibody was performed. **Results :** The mRNA level of TGF- β 1 in pulmonary valve was higher than those in the other valves. However, the protein levels of TGF- β 1 were similar among valves. The mRNA and protein levels of TGF- β 1 in cardiac valves were higher than those in atria or ventricles. The TGF- β 1 protein was located mainly in cellular interstitium in cardiac valves. The distribution of TGF- β 1 protein in surface area was higher than in the mid-portion of valves. **Conclusion :** These results suggest that synthesis and distribution of TGF- β 1 in cardiac interstitium is essential for maintaining of normal structure and function on various physical loading. (Korean Circulation J 1998;28(7):1161-1167)

KEY WORDS : Cardiac valves · TGF- β 1 · Physical loading.

서	론	peptide	¹⁻²⁾
TGF -	25 kd	TGF -	(multifunctional) 가 cy -
	가 homodimeric	tokine	
			³⁾
: 1998 4 9		, matrix	integrin adhesion
: 1998 6 25			, protease
: , 560 - 180,	2 - 20	protease inhibitor	, my -
: (0652) 270 - 3080 ·	: (0652) 270 - 4071	ogenesis	,
E - mail : gykoh@moak.chonbuk.ac.kr			

cytokine Northern Blotting¹³⁾

matrix (pulverization) TRI - reagent(MRC ,)

plasticity total RNA . Total RNA 20 µ

가⁴⁾⁵⁾ somatic 1.2% agarose gel , Gene Scr -

gene therapy⁶⁾ , een(New England Nuclear ,) 20 × SSC (sodium salts citrate)

TGF - 1 membrane UV crossl -

inker(Stratagene ,) RNA me -

membrane cross linking . Membrane hy -

bridization rotater 65

prehybridization probe

가 가 12 16 incubation

, TGF - 1 probe sense 5' - AT

TGF - 1 GCCGCC - TTCGGGGCCTGGGCTC(447 470)

1 TGF - antisense 5' - TCAGCAGCACTTGCAGGAACGC(16

01 1622) primer¹⁴⁾ -

(reverse transcriptase - polymerase chain re -

action) . Probe la -

belling random priming .

Incubation membrane

autoradiography .

Northern blotting Western blotting

TGF - 1

Western blotting 분석¹³⁾

(pulverization)

protease inhibitors가 1% NP - 40 detergent

2 ml 4 Polytron homoge -

nizer . sample 4 , 14,000

rpm Bradford

protein spectrophotometer

50 µ protein re -

ducing sample buffer 100

5 가 . 가 12% SDS pol -

yacrylamide gel nitrocellulose me -

membrane . Membrane 3% BSA, 5%

sampling nonfat dry milk, 0.1% Tween 20가 PBS

(phosphate buffered saline) , blocking

1 incubation .

(clone V ; Santa Cruz Biotechnology ,)

1 : 500 2 incubation pero -

재료 및 방법

실험동물과 심장판막 sampling

ICR

Sprague - Dawley .

25

10% neutral formalin

TGF - 1

10% neutral formalin

oxidase가 conjugation 1
incubation . ECL(Electroc -
hemiluminescence, Amersham ,)

면역조직화학법¹⁵⁾

TGF- β 1 10% neural
buffered formalin

Bouins 6

. Paraffin 5 μ m

xylene ethanol paraffin

30 0.6% H₂O₂가

methanol slide peroxidase

block . 10% goat serum block ,

TGF- β 1 1 : 50 1%,

BSA(bovine serum albumin)/PBS 4

overnight incubation .

anti-CC-1-30 (NCI/NIH

) TGF- β 1 1

30 peptides polyclonal ,

TGF- β

1 .¹⁶⁾ Biotin conjugation

1 incubation horsera-

dish peroxidase conjugation avidin incub-

ation . DAB(3,3'-diaminobenzidine

terahydrochloride) TGF- β 1 pro-

tein

결 과

심장판막에서 TGF- β 1 유전자 발현 및 단백질의 양적
인 비교

Fig. 1 , ma-

trix가 RNA

mobility . TGF- β 1 mRNA

transcript 1.9 kb 2.4kb 가

.¹⁷⁾ TGF- β 1 mRNA

(:

= 1 : 1.73 \pm 0.14, p<0.05). TGF- β 1

mRNA

2

, TGF- β 1

. Fig.

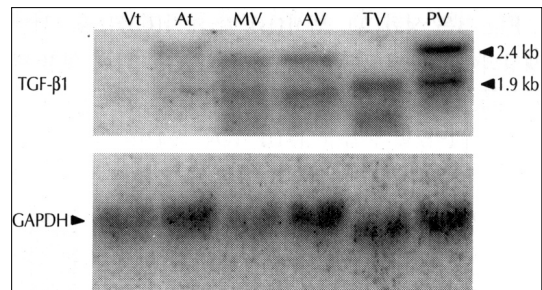


Fig. 1A. Northern blot analysis of TGF- β 1 in cardiac valves of adult pigs. Total RNA (20 μ g) from ventricles (Vt), atria (At) and four different cardiac valves (mitral valve, MV; aortic valve, AV; tricuspid valve, TV; pulmonary valve, PV) were examined. The radio-labeled TGF- β 1 probe was used for Northern blot, and the blot was hybridized with a GAP-DH probe to verify equal loading of RNA in each lane. Results were similar from three separate experiments.

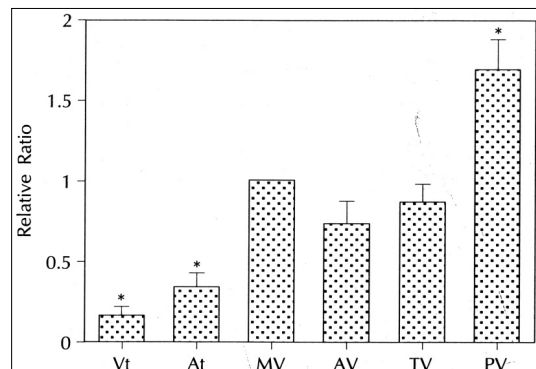


Fig. 1B. Densitometric analysis of TGF- β 1 mRNA in cardiac valves of adult pigs. To present relative mRNA level of TGF- β 1, densities of Northern blot signals were used. Densities of TGF- β 1 signal in mitral valve were presented as 1. Data are presented as mean \pm standard error of relative ratios from three separate experiments. The legends of cardiac valves are the same as shown in Fig. 1A.

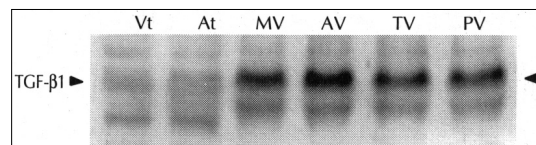


Fig. 2A. Western blot analysis of TGF- β 1 in cardiac valves of adult pigs. Total lysate protein (50 μ g) from ventricles, atria and four different cardiac valves were examined. The antibody used for TGF- β 1 was described in the METHODS section. Results were similar from three separate experiments.

1

TGF- β 1 동물종간에 따른 심장 승모판막에서 TGF- β 1의 조직학적 분포

Fig. 3 (A), (B) (C)
(A) TGF- β 1

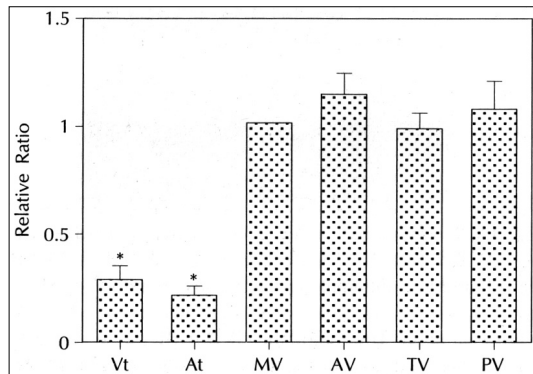


Fig. 2B. Densitometric analysis of TGF- β 1 protein in cardiac valves of adult pigs. To present relative protein level of TGF- β 1, densities of Western blot signals were used. Densities of TGF- β 1 signal in mitral valve were presented as 1. Data are presented as mean \pm standard error of relative ratios from three separate experiments. The legends of cardiac valves are the same as shown in Fig. 1A.

TGF- β 1

TGF- β 1가

TGF- β 1

고 찰

TGF- β 1

TGF- β 1

TGF- β 1 mRNA

TGF- β 1

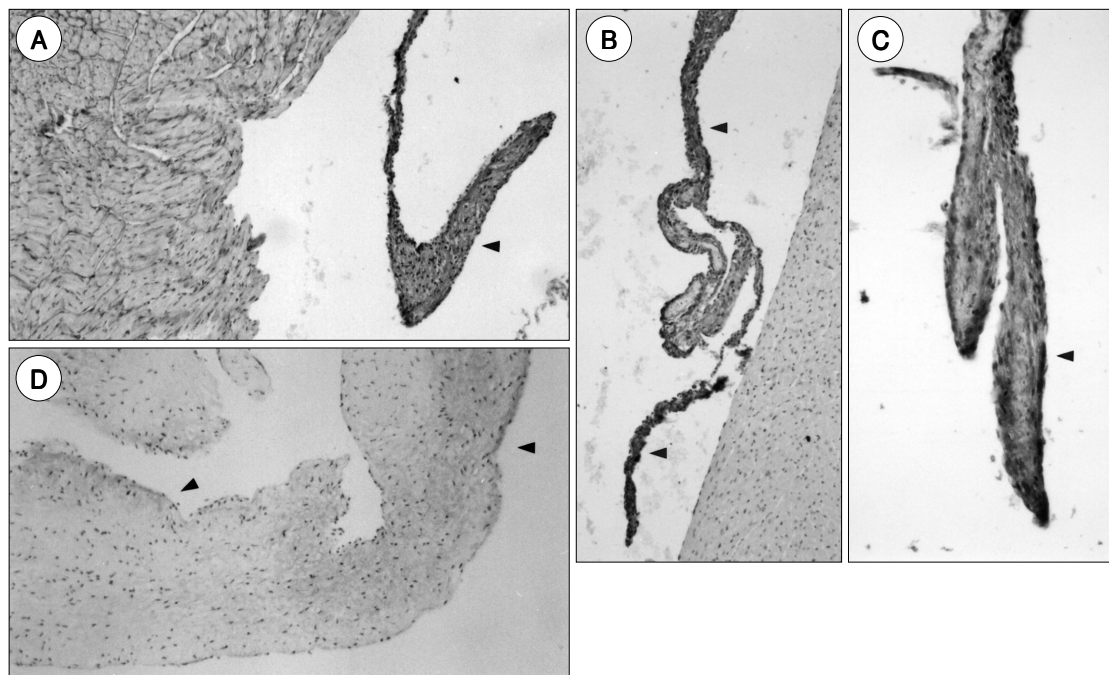


Fig. 3. Light micrographs of mitral valve in mouse (A), rat (B, C) and pig that were immuno-stained with TGF- β 1 antibody. Arrow heads indicate mitral valve. Detection of TGF- β 1 protein in valves was obvious, and appeared as intense dark-brown stained. Magnifications : A and D, $\times 100$; B, $\times 40$, C, $\times 200$.

. TGF -
 1 TGF - 1 가
 TGF - 1 matrix²⁰⁾
 가
 TGF - autocrine TGF - 1
 paracrine
⁸⁾⁹⁾¹²⁾
 atrioventricular
 canal (endothelial cell) 가
 (epithelial - mesenchymal transf - TGF - 1
 ormation) 68
 , 10 , 21 TGF - 1
 TGF -
 antisense
¹⁰⁾¹¹⁾ TGF - TGF - 1 가 ,
 TGF - 1 TGF - 1
 TGF - 3가 가¹¹⁾ 가
 Doetschman targeted gene disru - TGF -
 ption TGF - 1 (TGF - 1 가
 1 knockout)
¹⁸⁾ ,
 가
²¹⁾ TGF - 1 auto -
 crine paracrine
 TGF - 1
¹⁹⁾
 TGF - 1 TGF - 1
 ,
 , atrioventricular junctions,
 TGF - 가
¹⁹⁾
 TGF - 1²²⁾ TGF - 1
 가 가
 TGF -
 1가 TGF - 1
 TGF - 1
 matrix

연구배경 :

결 론 :

TGF- β

(multifunctional) 가 cytokine .
TGF- β 1 가

TGF- β 1 matrix

중심 단어 : TGF- β 1 .

감사문

(961-0701-003-

2)

TGF- β 1

TGF- β 1

TGF- β 1

대상 및 방법 :

(ICR), (Sprague-Dawley)

TGF- β 1
TRI-reagent

total RNA

Northern blotting TGF- β 1

pro-

tease inhibitors가 1% NP-40 detergent

Western

blotting TGF- β 1
anti-CC-1-30

DAB

결 과 :

1) TGF- β 1 mRNA

TGF- β

1 mRNA

TGF- β 1

TGF- β 1

2)

1

TGF- β

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