

# AT-1 심근세포를 대상으로 자발적인 박동을 나타내는 전·후단계에서 수종의 세포주기 조절 유전자의 발현에 관한 연구

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## Expression of Several Cell Cycle Regulatory Genes in Cultured AT-1 Cardiomyocytes after Formation of Synchronously Beating Sheets

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### ABSTRACT

**Background and Objectives :** AT-1 cells have been derived from the left atrial tissue in which the ANF promoter targeted SV40 large T antigen expression. When cultured, clusters of spontaneously contracting cells were observed after 4 -5 days and contiguous sheets of synchronously beating cardiomyocytes were formed after 10 days. In this study, expression of several cell cycle regulatory genes were monitored through Northern blot analyses in AT-1 cells during beating and after formation of beating sheets (BS). **Materials and Method :** AT-1 RNAs were obtained in 3 days after plating, during beating and after formation of BS, and used for Northern blot analyses. **Results :** -Cardiac myosin heavy chain expression was prominent in beating cells, as would be expected for this contractile protein isoform but ANF was decreased after beating. Gax was not expressed in cultured AT-1 cells but in AT-1 tumor and murine heart. *p53* and *p21* were decreased after beating which indicate transcription level of *p53* and *p21* correlated well in AT-1 cells. In contrast, *pRB* and *p107* were increased after beating but *p68* (2.4 kb) which arose by alternative splicing of *p107* and lacks the pocket domain B was decreased in beating cells. *pTCS2*, murine tuberous sclerosis gene, represented similar levels during beating but a little was decreased after formation of BS. *mRAD50*, the murine homologue of yeast DNA recombinational repair gene *RAD50*, was increased in beating cells, a similar pattern to *p107* and *pRB*. But the *p50* arose by alternative splicing of *mRAD50* and has 3' half of *mRAD50* had unexpectedly appeared and maintained after beating. **Conclusion :** The expression of cell cycle regulatory genes after beating and formation of BS in AT-1 cells showed gene-specific pattern and the *p50* which has homology to the *mRAD50* may participate in differentiation of cardiomyocytes. (Korean Circulation J 1998;28(4):611-619)

**KEY WORDS :** Cardiomyocyte · Cell cycle regulatory gene · Cardiomyocyte differentiation.

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## 서론

product) pRB · TAG . p53  
p53

AT - 1 atrial natriuretic factor(ANF) .<sup>5)</sup> p107 (fibroblast) TAG  
promoter simian virus 40 <sup>6)</sup> pRB pocket  
large T antigen(TAG) domain (homology) 가 <sup>7)8)</sup>  
transgenic mouse(ANF - TAG) pRB 가 가  
<sup>1)</sup>

histocompatible . p193  
mice(syngeneic host) 가 가 Daud <sup>4)</sup>  
(transplantable tumor)  
TAG p53  
(C - terminal) epitope 가 421  
IP 180 kDa  
DNA molecular cloning ,  
RAD50  
(N - terminal) 가  
rad50  
(transformation)  
가 RAD50  
homologue(mRAD50) <sup>9)</sup>

AT - 1 (cyto -  
kinesis) 96  
90% DNA trit - iated  
thymidine uptake . AT - 1  
- cardiac  
myosin heavy chain( - MHC), - cardiac actin,  
ANF AT - 1 가  
가  
<sup>2)</sup> Z density 가 (my -  
ofibril), ANF , T -  
tubule  
AT - 1  
<sup>3)</sup>  
4 5  
10  
TAG AT - 1 가  
가  
Daud <sup>4)</sup> TAG가  
TAG가  
가  
<sup>14)</sup>  
TAG  
TAG (immunoprecipitation, IP)  
TAG (target)  
p53, p107 p193 TAG  
(retinoblastoma gene

product) pRB · TAG . p53  
p53

(fibroblast) TAG  
<sup>6)</sup> pRB pocket  
<sup>7)8)</sup>  
가 가 가

histocompatible . p193  
Daud <sup>4)</sup>  
TAG p53  
(C - terminal) epitope 가 421  
IP 180 kDa  
DNA molecular cloning ,  
RAD50  
(N - terminal) 가  
rad50  
(transformation)  
가 RAD50  
homologue(mRAD50) <sup>9)</sup>

(cyto -  
kinesis) 96  
90% DNA trit - iated  
thymidine uptake . AT - 1  
- cardiac  
myosin heavy chain( - MHC), - cardiac actin,  
ANF AT - 1 가  
가  
<sup>2)</sup> Z density 가 (my -  
ofibril), ANF , T -  
tubule  
AT - 1  
<sup>3)</sup>  
4 5  
10  
TAG AT - 1 가  
가  
Daud <sup>4)</sup> TAG가  
TAG가  
가  
<sup>14)</sup>  
TAG  
TAG (immunoprecipitation, IP)  
TAG (target)  
p53, p107 p193 TAG  
(retinoblastoma gene

## 재료 및 방법

### RT-PCR을 이용한 cDNA probe 제작

Reverse transcriptase - polymerase chain reaction (RT - PCR) cDNA probe

Cardiac myosin heavy chain ( - MHC), sense primer 5' - CTG - CTGGAG - AG - GTTATCCTCG - 3' antisense primer 5' - GGAAGAGTGAGCGGCGCACAAGG - 3' band 302 bp .

Atrial natriuretic factor (ANF) sense primer 5' - CGTGCC - CC - GACCCACGCCAGCATGG - CCC - 3' antisense primer 5' - GGCTCCGAGGGCCAGCGAG - CA - AGCCTCA - 3' band 389 bp .

Gax, sense primer 5' - AGCCTGATT - CCGGAGGGCCCCCGGAGCTGG - 3' antisense primer 5' - TGCAGCTCCTTGTTGTCCCC - CC - TTGACCCG - 3' band 428 bp .

p53, sense primer 5' - GAGGAGTC - TGGAGACA - GCAGGGCTCACTCC - 3' antisense primer 5' - TGAGATTTTCATTGTAGGTG - CCAGGG - TCCAA - 3' band 521 bp .

p21, sense primer 5' - TCTTG - TG - TTTTCAGCCACAGGCACCATGTCC - 3' antisense primer 5' - TCCTTCTCTGCTCCTG - TCC - TTACCTGTCTAG - 3' band 477 bp .

pRB, sense primer 5' - TC - TGCA - AGTGATCAGCCATCAGAAAATCTG - 3' antisense primer 5' - AAGTACGTTCA - GAATCCA - CGGGAAGGACAA - 3' band 393 bp .

p107, sense primer 5' - ATACTA - AAAGGAATAGGAGAGACTTT - CTGT - 3' antisense primer 5' - CACCAT - GTCCCTTGAGAG - CCCCTCTTCTGA - 3' band 369 bp .

pTSC - 2 sense primer 5' - GCCAC - AGCCAGCATGGCCGTCC - CACTGCTG - 3' antisense primer 5' - GG - ACGTCTGTATCCTG - CTGCGGACCACATG - 3' band 497 bp .

p153 (mRAD -

50) , sense primer 5' - ATGACATAGA - AGAGCAGGAAACACTCTTG - G - 3' antisense primer 5' - TTATCCTGTAG - CCACCTT - TCCTGTATCTTC - 3' band 766 bp .

50 200 ng AT - 2 RNA 100  $\mu$ l reaction buffer (50 mM KCl, 10 mM Tris - HCl pH 8.3, 2.5 mM  $MgCl_2$ , 0.25 mM dNTP 0.01 mg/ml gelatin) 0.3  $\mu$ g antisense oligonucleotide primer, 0.2  $\mu$ g sense oligonucleotide primer, Taq polymerase (2.5 units, Amplitaq, Perkin - Elmer Cetus Corp., Norwalk CT) 가 . 85 5 10 10 units reverse transcriptase (International Biotechnologies Inc., New Haven CT) thermocycler (Perkin Elmer) . 42 1 , 94 10 - MHC ANF 94 1 , 62 1 , 72 1 30 cycle 72 20 . Gax, p53, p21, pRB, p107, pTSC - 2, mRAD50 94 1 , 66 2 , 72 3 35 cycle 72 20 . agarose gel band TA vector cloning system (Invitrogen, San Diego CA) subcloning cDNA probe .

### 세포배양

AT - 1 Steinhilber<sup>15)</sup> TAG transgenic 4 14 0.125% trypsin 가 Joklik's minimum essential medium . 37 0.1% collagenase 가 가 Joklik's minimum essential medium 10 . 4 500 rpm 1 Joklik's minimum essential medium 3 4 . 10 collagenase  $0.5 \times 10^6$  cells/

ml collagen-coated T25 tissue culture flask  
 plating penicillin(100 U/ml), streptomycin(100  
 $\mu$ g/ml), 10% fetal calf serum, human insulin(10  $\mu$   
 g/ml), iron-free human transferrin(10  $\mu$ g/ml), 1%  
 chick embryo extract, 10 nM dexamethasone  
 PC - 1 2 3

#### RNA 제작 및 Northern Blot 분석

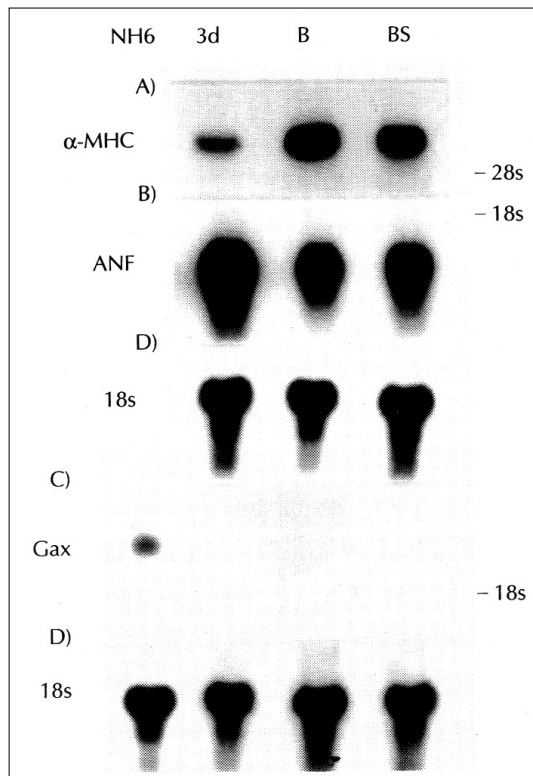
AT - 1 3 ,  
 , 10  
 3  
 total RNA , 4 1  $\times$  phosp -  
 hate - buffered saline 3  
 1% -mercaptoethanol 가 4M guanid -  
 ium thiocyanate 2 ml polytron  
 4 10,000 rpm 5 1  
 ml cesium pad solution(5.7 M CsCl) overlay  
 40,000 rpm 20 8  
 (Beckman TL100). tube  
 RNA diethylpyr -  
 ocarbonate ethanol pre -  
 cipitation 260 nm spectrophotometer

Northern 10  $\mu$ g total RNA 2  
 glyoxal 56 20 denature  
 1.0% agarose gel 3 running Ge -  
 nescreen(DuPont, Wilmington DE) 12  
 transfer . Probe random primer method(Pr -  
 omega, Madison WI) Nick translation kit(Boer -  
 inger Mannheim, Indianapolis IN)  
 2 3  $\times 10^9$  dpm/ $\mu$ g specific ac -  
 tivity . Hybridization 65 4  $\times$  SSC,  
 2  $\times$  Denhardt's, 0.1% SDS 100  $\mu$ g/ml salmon sp -  
 erm DNA 12 washing  
 65 2  $\times$  SSC, 0.1% SDS 1  
 . intensifying screen cassette  
 film - 70  
 . blot 18s rRNA oligonucleotide  
 probe(5' - TCCATTATTCCTAGT - GCGGTAT -  
 CCAGGAGGATCGGGCCTGCTTT - 3') No -

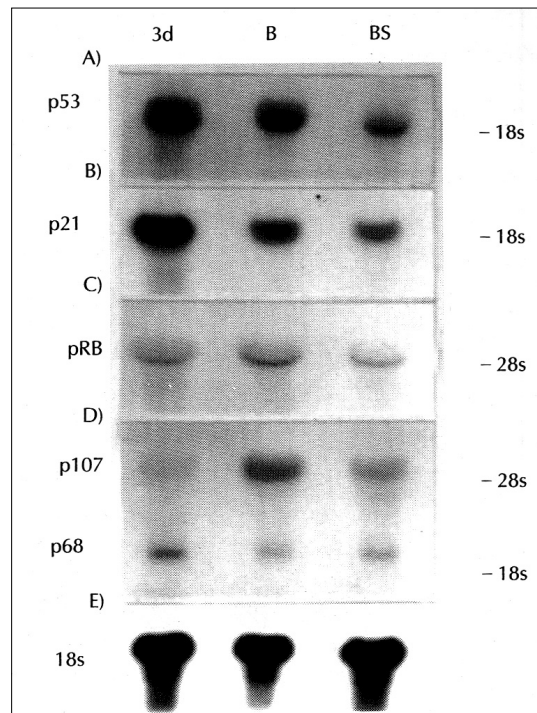
rthern

#### 결 과

AT - 1 .  
 Northern blot .  
 - MHC, ANF  
 homeobox gene  
 Gax Fig. 1 . -  
 MHC  
 AT - 1  
 . ANF  
 . Gax AT - 1  
 . Gax AT - 1 ( )  
 . AT - 1  
 RNA  
 18s rRNA oligonucleotide  
 blot reprobing Fig. 1 .  
 18s rRNA  
 가 p53 pRB, p53 descending  
 mediator p21, pRB pocket domain  
 가  
 p107 Fig. 2 .  
 p53 . p21  
 . pRB  
 가 . p107  
 Northern blot band 4.9  
 kb (p119) 가  
 blot band 2.4 kb  
 (p68) 4.9 kb  
 . AT - 1  
 RNA Fig. 1  
 (Fig. 2 ).  
 pTSC2 DNA



**Fig. 1.** Northern blot analysis of cardiac-specific gene expression in different stages of cultured AT-1 cardiomyocytes. Parallel blots with total RNA from AT-1 cells in different stages were examined. Total RNA was isolated from 3 days (3d) culture, after appearance of cluster of contracting cells (B) and after formation of broad contiguous sheet of synchronously beating cell (BS). In case of Gax, neonatal heart (6 days, NH6) RNA was included as control. The radiolabeled cDNA probes used for each blot were: MHC (panel A), ANF (panel B) and Gax (panel C). Blots were also probed with a radiolabeled oligonucleotide complementary to mouse 18s rRNA (panel D), to confirm the integrity and quantity of the samples. The positions of the 18s and 28s ribosomal RNA bands are indicated on the right side.



**Fig. 2.** Northern blot analysis of tumor suppressor gene expression in different stages of cultured AT-1 cardiomyocytes. Parallel blots were probed with the following mouse cDNAs: p53 (panel A), p21 (panel B), pRB (panel C) and p107 (panel D). Blots were also probed with a radiolabeled oligonucleotide complementary to mouse 18s rRNA, to confirm the integrity and quantity of the samples (panel E).

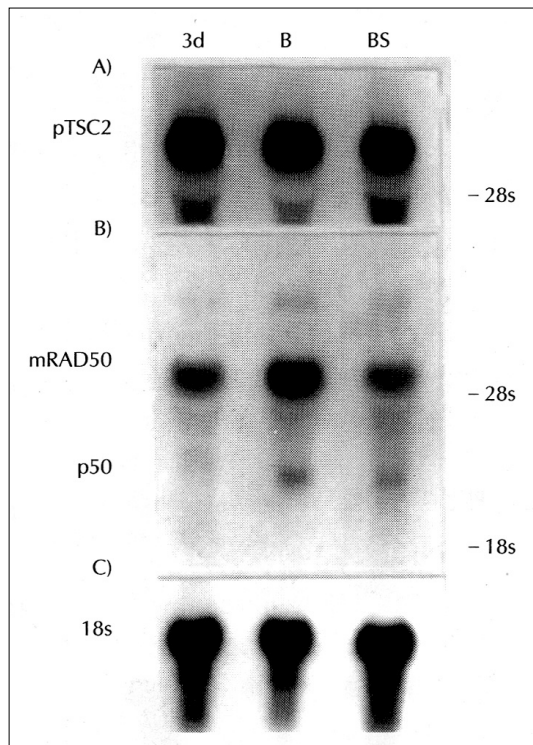
AT - 1 RNA  
Fig. 1  
(Fig. 3).

## 고 찰

mRAD50  
. pTSC2  
Fig. 3  
가  
mRAD50, Northern blot  
band 5.1 kb  
가  
band 2.6 kb  
(p50)

Daud <sup>4)</sup> TAG (AT -  
1, AT - 2) TAG p53, p107,  
p193 TAG p53  
epitope 가  
180 kDa (mRAD50)  
Kim <sup>16)</sup> AT - 1 AT - 2 pRB가  
TAG Western blot

hypophosphorylated form



**Fig. 3.** Northern blot analysis of *pTSC2* and *mRAD50* gene expression in different stages of cultured AT-1 cardiomyocytes. Parallel blots were probed with *pTSC2* (panel A) and *mRAD50* (panel B). Blots were also probed with a radiolabeled oligonucleotide complementary to mouse 18s rRNA, to confirm the integrity and quantity of the samples (panel C).

TAG pRB  
 p53  
 p53  
 p53  
 Kim <sup>16)</sup> p53  
 (descending mediator)  
 p21<sup>18)</sup> p53  
 p21 p27, p16, p15  
 cyclin - dependent kinase inhibitor(CdKI)  
 CdK proliferating cell nu -  
 clear antigen DNA E2F  
 complex <sup>19)</sup>  
 p21 p53 <sup>18)</sup>  
 p21 DNA p53

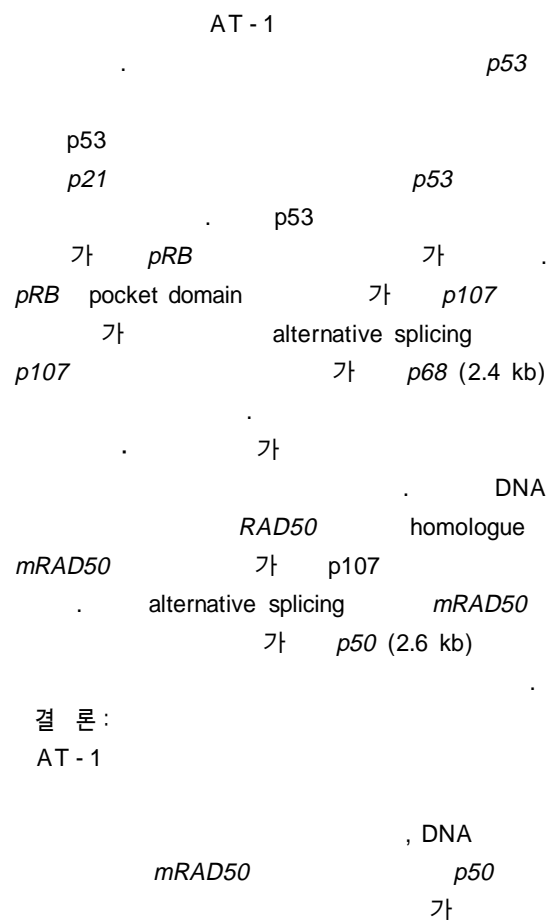
transforming growth factor  
 가  
 p21 p53  
 가  
 mRNA  
 가  
 pRB AT - 1  
 가  
 pRB Kim <sup>16)</sup> pRB  
 p53  
 p53 p21 가  
 pRB TAG p53  
 TAG p53  
 pRB  
 pocket domain family p107  
 4.9 kb(p119)  
 가 alternative splicing po -  
 cket domain B7 가 2.4 kb(p68)  
 p119  
 가 pRB  
 mRNA  
 p107 mRNA  
 TAG E1A  
 pocket domain B7 가  
 p119  
 가  
 pTSC - 2  
 가  
 pTSC - 2  
 가 Kim <sup>21)</sup> 가  
 pTSC - 2 가  
 , pTSC - 2 가  
 TAG  
 가  
 pTSC - 2  
 AT - 1 pTSC - 2 가

*RAD50* homologue *mRAD50*<sup>9)</sup>  
 가 *pRB* p119  
 alternative splicing  
*mRAD50* (5.1 kb)  
 가 p50(2.6 kb)  
 Gax  
 homeobox  
 platelet-derived growth factor  
 serum growth factor  
*Gax* mRNA level  
 가 *Gax*가  
 가  
 cardiac myosin binding protein - c가  
 (thick filament)  
 (assembly)<sup>22)23)</sup>  
 cAMP-dependent protein kinase calmodulin-dependent protein kinase  
 AT - 1  
 Gax가  
 가  
 p50<sup>24)</sup>  
 molecular cloning  
 9)

요약

연구배경 :  
 AT - 1 ANF promoter SV40  
 large T antigen tran -  
 sgenic mouse  
 4 5  
 10  
 ANF  
 c - fos  
 가<sup>25)</sup>  
 AT - 1 3 ,  
 3  
 Mantymaa<sup>26)</sup>  
 ANF brain natriuretic  
 peptide(BNP) 가 가 BNP  
 가 ANF  
 McDonough Glembo -  
 tski<sup>27)</sup>  
 ANF myosin light chain -  
 2 가 ,  
 nifedipine W7 ANF 가  
 ANF  
 TAG AT - 1  
 homeobox  
 Gax AT - 1

방 법 :  
 AT - 1 RNA  
 cDNA probe RT -  
 Northern  
 PCR  
 blot  
 결 과 :  
 - cardiac myosin heavy chain  
 . ANF



중심 단어 :

감사문

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