

본태성 고혈압쥐에서 Myogenic Tone의 변화 : RhoA 단백질 및 Protein Kinase C의 역할

BK21, 1, 2
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Changes in Myogenic Tone in Spontaneously Hypertensive Rat : Role of RhoA and Protein Kinase C

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ABSTRACT

Background and Objectives : The myogenic response was originally described as a contraction of a blood vessel that occurred following an increase in intravascular distending pressure. Conversely, a reduction in intravascular pressure produces myogenic vascular relaxation. Recent attention has focused on the potential role of this myogenic mechanism in the control of tone in the resistance vasculature, and in particular on how this mechanism may contribute to the increased vascular resistance seen in hypertension. Therefore, in the present study, we investigated the role of myogenic tone in the generation and/or maintenance of hypertension. **Materials and Methods** : Myogenic tone was developed by stretching of the basilar arteries of WKY (Wistar Kyoto rat) and SHR (spontaneously hypertensive rats). Contractile responses, PKC (protein kinase C) immunoblots and translocation of PKC and RhoA were measured. **Results** : In the presence of extracellular Ca²⁺, the stretching of the resting vessel evoked a myogenic contraction in the basilar arteries of SHR and WKY. Myogenic tone was significantly greater in SHR than in WKY. However, in the absence of extracellular Ca²⁺, stretching evoked a myogenic contraction in SHR, but not in WKY. The stretch-induced myogenic tone was inhibited by nifedipine. The effect of nifedipine was similar in both SHR and WKY rats. H-7, calphostin C and Y-27632, also inhibited stretch-induced myogenic tone in both SHR and WKY. The inhibitory effects of these drugs were greater in SHR than in WKY. Immunoblotting showed rho A and PKC were translocated from the cytosol to the cell membrane with stretching in both SHR and WKY. PKC, however, was translocated to the cell membrane with stretching in SHR, but not in WKY. **Conclusion** : These results suggest that stretch-induced myogenic tone is significantly greater in SHR than in WKY. Furthermore, the increase in amount and/or activity of PKC and ROK (rhoA-associated kinase) may be a key mechanism accounting for the enhanced myogenic tone in SHR. (**Korean Circulation J 2002;32(3):257-267**)

KEY WORDS : Myogenic tone ; Protein kinase C ; RhoA GTP-binding protein ; Rats, inbreds SHR.

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서 론

(perfusion pressure)
 (blood flow)
 가
 (autoregulation)¹⁾
 (myogenic), (neurogenic) (metab-
 olic)
 가 (myogenic response)
 가
 ,
³⁾ in vivo
 in vitro
 (stretch)
 가
 (myogenic tone)
 ,
 Ca²⁺ 가 가
⁴⁾ Ca²⁺ 가 1)
 stretch activated cation
 Ca²⁺ ,⁵⁾ 2) Ca²⁺
 Ca²⁺ ,⁶⁾ 3)
⁶⁾ Ca²⁺ 가
 ,
 PKC(protein kinase C)가
 Ca²⁺ (Ca²⁺ sensit-
 ization) 가 .⁷⁾
 small G - protein rhoA가 Ca²⁺
⁸⁾
 ,
 Ca²⁺
 , small G - protein rhoA
 ROK(rho - kinase)가 가
 MLCP(myosin light chain phosphatase) M₁₁₀₋₁₃₀

regulatory subunit
 activity
 rhoA/ROK
 Ca²⁺
 (basal vascular resi-
 stance)¹¹⁾
 가
 가
 가
 ,
¹²⁾
 pillary pressure)
 ,
 (SHR, spontaneously
 hypertensive rat) 가
¹³⁾
 가
 가
 ,
 Ca²⁺ 가 가
¹⁴⁾
 가
 ,
¹⁵⁾
 (myogenic constriction)
¹⁾ 가
¹⁶⁾ 가
 ,
 가
 ,
 aortic-
 coarctation - induced hypertension 가
 3 4
 PKC 가
¹⁴⁾
 ,
 SHR 가
 SHR
 1) SHR WKY(Wistar Kyoto rat)
 , 2) SHR WKY

재료 및 방법

실험재료

Charles River Japan (Yokohama, Japan) 12 SHR(; 200±12 mmHg) WKY(; 140±12.5 mmHg)

nifedipine, calphostin C, H - 7 western blot (St Louis, MO, USA) PKC rhoA Rho A Santa Cruz (Santa Cruz, California, USA) PKC Transduction Laboratory (San Diego, California, USA) . Y - 27632 We - lfide Corporation(Osaka, Japan)

실험방법

SHR WKY basilar artery bas - ilar artery 95% O₂+5% CO₂ Krebs - Henseleit (mM : NaCl 119, KCl 4.6, CaCl₂ 2.5, KH₂PO₄ 1.2, MgSO₄ 1.5, NaHCO₃ 25, glucose¹¹) preparation chamber

가 helical st - rip 가 37 Krebs - Henseleit 4 mN 가 1 가 50.5 mM K⁺ (Kr -

ebs - Henseleit KCl 가 50.5 mM NaCl) 10⁻⁶ M acetylcholine

1 he - lical strip 50% 가 Helical strip () Ca²⁺ nifedipine, H - 7, calphostin C Y - 27632

Western blot

SHR WKY basilar artery dry ice/acetone 50 mM Tris(pH 7.4), 10% glycerol, 5 mM EGTA, 140 mM NaCl, 1.0% Nonidet P - 40, 5.5 mM Le - upeptin, 5.5 mM pepstatin, 20 KIU aprotinin, 1 mM Na₃VO₄, 10 mM NaF, 0.25%(wt/vol) sodium deo - xycholate, 100 μM ZnCl₂, 20 mM - glyceroph - osphate, 20 μM phenylmethylsulfonyl fluoride가 buffer homogenization . Protein - matched sample(30 μg protein/lane) 10% SDS - polyacrylamide gel Millipore Im - mobilon - P membrane transfer . Membrane 5% dried milk가 PBS - Tween buffer 1 (room temperature) incubation pr - imary antibody, PKC (1 : 500 ; Transduction La - boratory), PKC (1 : 500 ; Santa Cruz), Rho A (1 : 200 ; Santa Cruz) 4 overnight incubation . membrane horseradish peroxidase - conjugated secon - dary antibody(1 : 10,000 ; Calbiochem) 1 incubation . Immunoreactive band enhanced chemilumi - nescence(ECL ; Am - ersham) . ECL scan PKC isoform RhoA TINA 2.0 program(Ray - test ; Germany) Fuji Photo Film Image X - ray film

densitometry

PKC isoform rhoA translocation

PKC PKC rhoA

SHR WKY

western blot

protein - matched sample immunoblot

western blot

artery

8 basilar 통계처리

50.5 mM K⁺

가 mean ± SE

incubation strip , " n "

liquid N₂ - cooled liquid strip

chlorodifluoromethane homog - Student t - test

enization {200 mM Tris - HCl(pH 7.4), 0.3 M sucrose, 5 mM EDTA, 5 mM DTT, 10 mM EGTA, 0.3 mM phenylmethylsulfonyl fluoride, 0.3% 2 - mercaptoethanol} homogenization . Ho - hom -

ogenates 100,000 g(4) 60

0.1% Triton X - 100 가 SHR WKY

결 과

SHR과 WKY의 basilar artery에서 신장이 장력에 미치는 영향

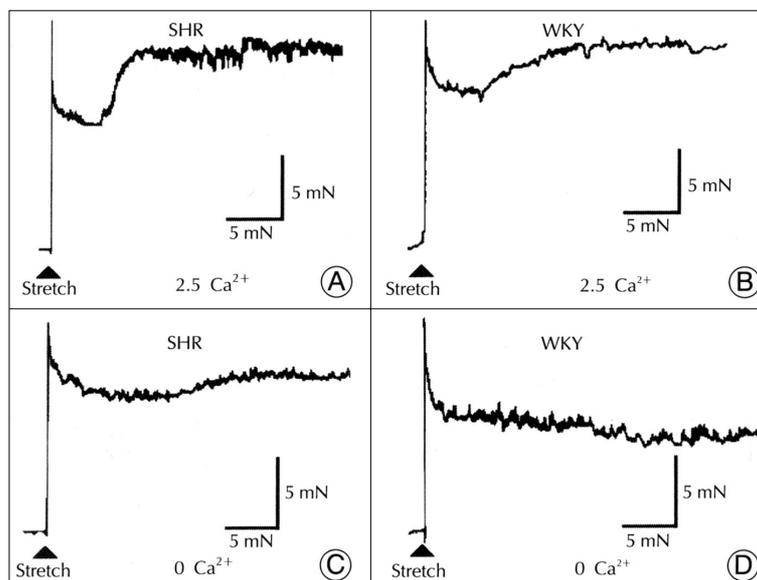


Fig. 1. Typical records of myogenic tone induced by stretch in basilar artery of SHR and WKY. A and B : typical record of changes in tension by stretch in the presence of extracellular Ca²⁺ (2.5 mM Ca²⁺ ; 2.5Ca²⁺). C and D : typical record of changes in tension by stretch in the absence of extracellular Ca²⁺ (0Ca²⁺). Helical strips were stretched passively to the optimal length by imposing a stretch of 50% of resting length. SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

basilar artery

. Fig. 1

가

SHR WKY

5 mM K^+

38.2 ± 6%

62.1 ± 12.2%

WKY

SHR WKY

2.5 mM Ca^{2+}

(passive tension)

SHR WKY

WKY

(Fig. 1).

SHR

15 ± 1.2% (Fig. 2 ; n=14)

Ca²⁺

Fig. 2 SHR 50.

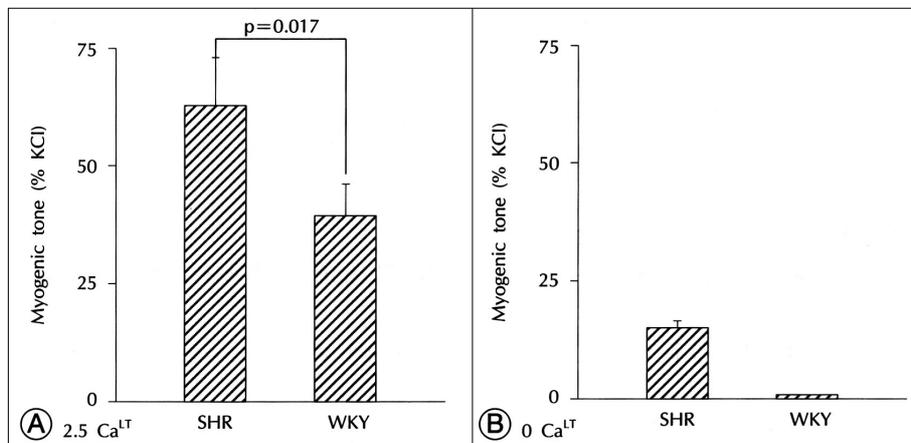


Fig. 2. Statistical analysis of the amplitude of myogenic tone induced by stretch between SHR and WKY. A : comparison of amplitudes of myogenic tone between SHR and WKY in the presence extracellular Ca^{2+} (2.5 mM Ca^{2+} ; 2.5 Ca^{2+}). B : comparison of amplitudes of myo-genic tone between SHR and WKY in the absence of extracellular Ca^{2+} (0 Ca^{2+}). Data are expressed as relative percentage of 50.5 mM high K^+ response. Results are expressed as mean ± SE (n=14). SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

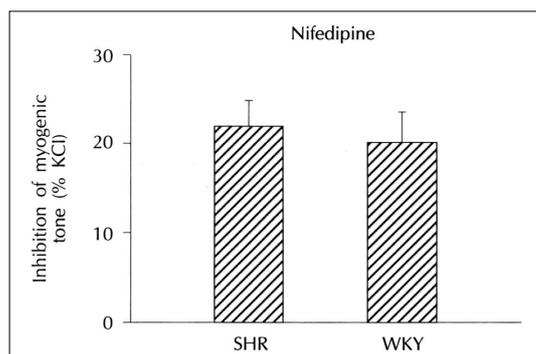


Fig. 3. Effect of nifedipine on the stretch-induced myogenic tone. Nifedipine (10^{-7} M) was added when stretch-induced myogenic tone was stable. Data are expressed as relative percentage of 50.5 mM high K^+ response. Results are expressed as mean ± SE (n=8). SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

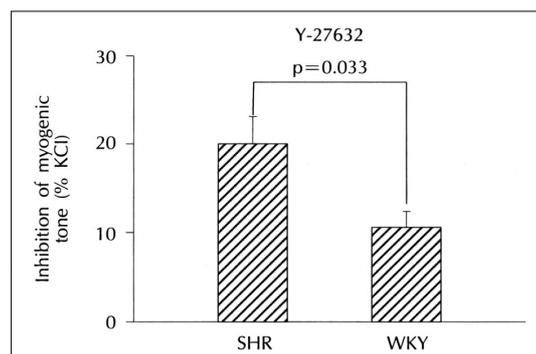


Fig. 4. Effect of Y-27632 on the stretch-induced myogenic tone. Y-27632 ($1 \mu M$) was added when stretch-induced myogenic tone was stable. Data are expressed as relative percentage of 50.5 mM high K^+ response. Results are expressed as mean ± SE (n=9). SHR : spontaneously hyper-tensive rat, WKY : Wistar Kyoto rat.

Nifedipine이 신장에 의한 장력 변화에 미치는 영향

10⁻⁶
 M Y - 27632 Y - 27632 SHR
 Ca²⁺ nife -
 dipine (Fig. 3). SHR WKY
 가가 (p=0.033)
 10⁻⁷ M nifedipine
 nifedipine
 SHR 23.2 ± 2.3%(n=8), WKY 20.2 ± 3.6%
 (n=8) SHR WKY 가 .
 5 × 10⁻⁷ M calphostin - C
 5 SHR WKY

Rho-kinase와 PKC 억제제가 신장에 의한 장력 변화에 미치는 영향

rhoA
 PKC가 rho - kinase
 Y - 27632(Fig. 4) PKC H - 7 ca -
 lphostin C(Fig. 5) . , SHR
 WKY small G - protein rhoA
 rho - kinase Y - 27632
 (Fig. 4). SHR WKY
 SHR (H - 7 ; p=0.0335, calphostin
 C ; p=0.0344)
 H - 7 calphostin - C
 가 12.2 ± 2.2% 12.3 ± 3.1%(n=8)
 , WKY 5.3 ± 2.1% 6.3 ± 1.7%(n=8)

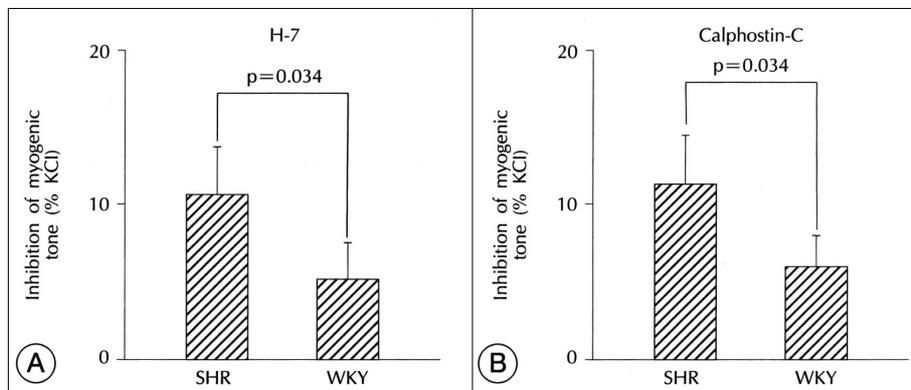


Fig. 5. Effect of H-7 and calphostin-C on the stretch-induced myogenic tone. H-7 (10⁻⁵ M) and calphostin-C (5 × 10⁻⁷ M) was added when stretch-induced myogenic tone was stable. Data are expressed as relative percentage of 50.5 mM high K⁺ response. Results are expressed as mean ± SE (n=8). SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

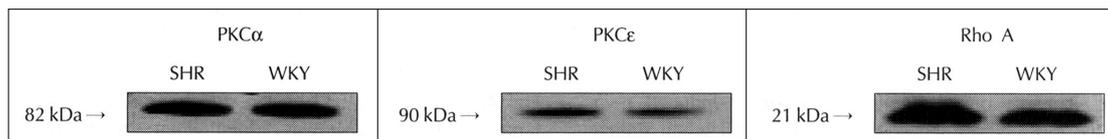


Fig. 6. Immunoblots of PKC-α, -ε, and rhoA in isolated basilar artery. Immunoblots are representative of five independent preparations. SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat, PKC α : protein kinase C alpha, PKC ε : protein kinase C epsilon.

신장이 rhoA 단백질과 PKC의 translocation에 미치는 영향

SHR WKY basilar artery rhoA
 PKC isoforms rhoA
 PKC isoform specific antibody western blot

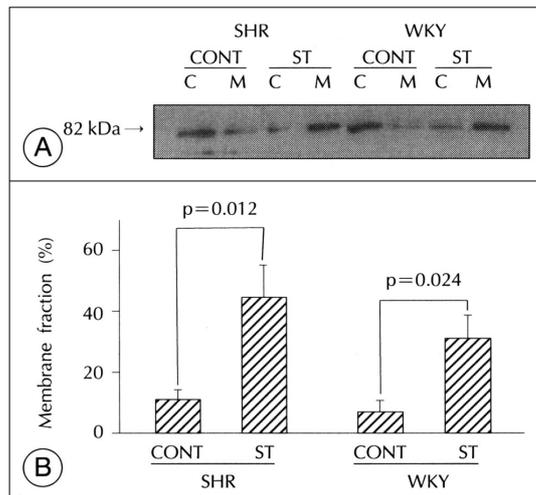


Fig. 7. Stretch-induced translocation of rhoA. A : typical data for stretch-induced translocation of rhoA. Results are representative of five experiments showing that rhoA is translocated from the cytosol (C) to the membrane (M) fraction by stretch (ST) in both SHR and WKY. B : statistical analysis for changes in membrane fraction by stretch in both SHR and WKY. Results are expressed as mean \pm SE. CONT : non-stretched tissues. SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat.

Fig. 6

western blot PKC
 band
 SHR WKY
 , PKC rhoA WKY SHR
 band .
 rhoA가 trans-
 location SHR WKY Fig. 7
 rhoA가 가
 , SHR rhoA 가
 46.3 \pm 9.6% (p=0.0123, n=5)
 , WKY 11.0 \pm 2.9%
 9% 32.1 \pm 7.1% (p=0.0237, n=5)
 rhoA translocation 가 SHR
 PKC isoforms translocation
 PKC (Fig. 8).
 PKC SHR WKY
 가
 SHR WKY

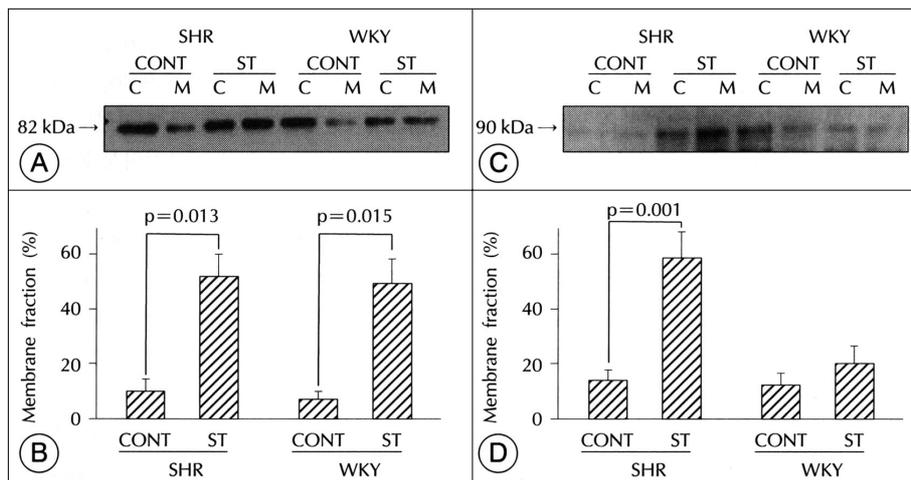


Fig. 8. Stretch-induced translocation of PKC (A) and PKC (C). Results are representative of five experiments. B and D : statistical analysis for changes in membrane fraction by stretch in both SHR (B) and WKY (D). Results are expressed as mean \pm SE. ST : stretched tissues, CONT : non-stretched tissues. SHR : spontaneously hypertensive rat, WKY : Wistar Kyoto rat. C : cytosol, M : membrane.

10.4 ± 4.7% 8.3 ± 2.2% (n=5)

52.6 ± 7.4%

50.2 ± 9.1% (n=5) PKC tr-
anslocation (SHR ; p=0.0128, WKY ; p=
0.0149) 가 SHR WKY PKC tra-
nslocation

PKC translocation SHR SHR WKY 20
가, 가

14.3 ± 3.9% (n=5), 가 SHR WKY 20)
59.3 ± 10.1% (n=5) translocation SHR mesenteric artery 가
(p=0.0007) 가 , WKY 12.5 ± 4.2% (n=5) 21) 가
19.5 ± 5.2% (n=5) 가 SHR 17)18)

고 찰

가 , aortic coarctation
Ca²⁺

가 22)

가 Ca²⁺

가 SHR 가 Ca²⁺ 가

1) SHR WKY Ca²⁺ nifedipine

가 16) Ca²⁺ nifedipine

가 SHR nifedipine

가 SHR WKY SHR WKY

SHR WKY (stretch)

Ca²⁺ Ca²⁺ 가 SHR aortic coarctation 23)
WKY WKY Ca²⁺ 가 24)

SHR 가 Ca²⁺ 가 SHR WKY

SHR 가 가 nifedipine

가 SHR WKY 가 가

SHR cremaster 17) 18) nifedipine 가 가

가 가 10⁻⁵ M 25)

14) 가 10⁻⁷ M nifedipine 26)

가 가 nifedipine

SHR 가

Ca²⁺ WKY , PKC SHR

SHR 가 SHR 가

Ca²⁺ nifedipine isoform classical PKC(, 1, 2) Ca²⁺, diacylglycerol phosphatidylserine novel PKC(, ,) Ca²⁺ - independent isoform dia-cylglycerol phosphatidylserine

Ca²⁺ rhoA . rhoA Ca²⁺ - independent isoform dia-cylglycerol phosphatidylserine

Ca²⁺ rhoA . Atypical PKC(,) phosphatidylserine

Ca²⁺ rhoA . PKC SHR

Ca²⁺ rhoA . PKC

SHR 가 SHR 가

rho - kinase Y - 27632가 rhoA PKC translocation

WKY SHR rhoA DNA western blot rhoA

rhoA band가 WKY SHR PKC가 myosin light chain

SHR WKY 가 .¹⁰⁾ rhoA PKC가 myosin light chain phosphatase myosin light chain 가 .¹⁰⁾²⁸⁾

rhoA translocation 가 WKY SHR rhoA PKC

가 Ca²⁺ 가가 rhoA PKC

가 SHR 가 . G - protein phospholipase C , phospholipase C phosphatidylinositol - 4,5 - bisp - hosphate diacylglycerol PKC가

가 .²⁹⁾

SHR WKY

western blot PKC SHR WKY SHR 가 rhoA

band PKC band PKC 가 가

WKY SHR PKC가

PKC western blot PKC

SHR WKY translocation

tion PKC SHR PKC

translocation . PKC

¹⁰⁾

요 약

배경 및 목적 : (myogenic response)

가

가
 가
 etch)
 WKY
 방 법 :
 basilar artery (stretch)
 basilar artery
 , rhoA PKC immun-
 oblot translocation
 결 과 :
 Ca²⁺ basilar ar-
 tery
 SHR WKY
 WKY SHR
 Ca²⁺
 SHR
 WKY
 Ca²⁺ nifedipine SHR WKY
 kinase Y - 27632 SHR WKY
 WKY SHR
 PKC H - 7 calphostin - C
 WKY SHR . RhoA
 PKC isoforms - specific antibody im-
 munoblotting PKC SHR WKY
 band rhoA PKC WKY
 SHR band . RhoA
 SHR WKY 가
 translocation . translocation
 WKY SHR
 PKC SHR WKY
 translocation

PKC SHR translocation
 WKY
 결 론 :
 SHR WKY
 가 rhoA
 PKC 가 가
 중심 단어 : ; Protein kinase C ; RhoA
 ;

1999
 (: 1999 - 2).

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