

E1B-19k의 세포내 위치와 Bax와의 Dimerization에 관한 연구

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= Abstract =

E1B-19k does not Localize in Mitochondria nor Dimerize Bax even with the Staurosporine

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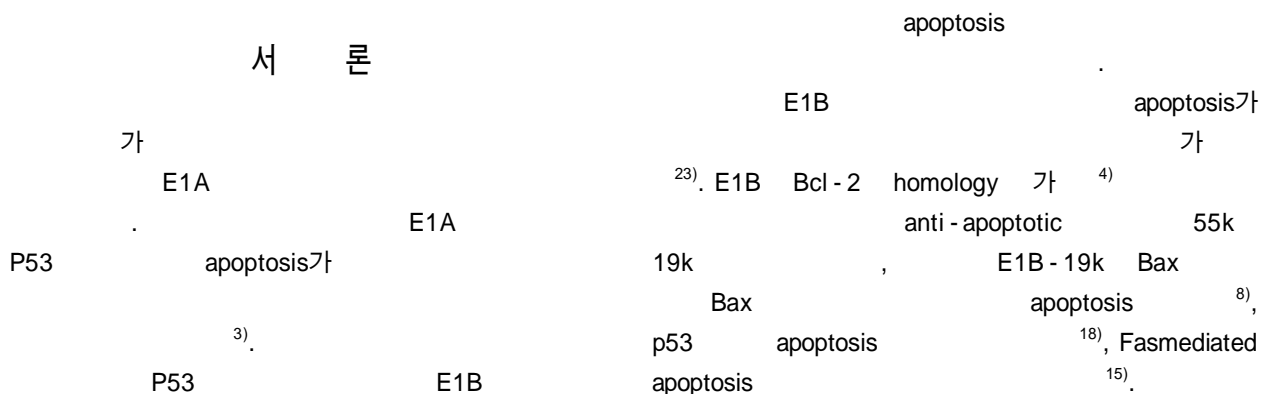
Purpose : The subcellular localization of E1B - 19k has been known cytosol or nuclear membrane by immuno - histochemical staining and could dimerize with Bax to regulate cell death also known by the *in - vitro* immuno - precipitation. We planed to confirm this dimerization of E1B - 19k with Bax in vivo in Cos - 7 cells by using green fluorescent protein.

Material and Method : We cloned E1B - 19k and Bax into C3 - EGFP. C3 - EGFP - E1B - 19k, C3 - EGFP - Bax, and C3 - EGFP - E1B - 19k and pcDNA3 - Bax were transfected into Cos - 7 cells. We explored location of E1B - 19k and Bax, and confirmed its dimerization with Bax in transfected living healthy Cos - 7 cells by following green fluorescent protein of E1B - 19k on the confocal microscope.

Results : E1B - 19k was located diffusely in cytoplasm and in nucleus but not in mitochondria. It prevented cell death from the apoptosis by staurosporine but its location was not changed. GFP - E1B - 19k is not changed its intracellular location with Bax even with staurosporine.

Conclusion : These results support that E1B - 19k does not localize in mitochondria nor dimerize with Bax even with staurosporine. We could anticipate E1B - 19k prevent cell death via the other dimerizing partner or pathways.

KEY WORDS : E1B - 19k · Bax · Dimerization · Apoptosis · Staurosporine · Green fluorescent protein.



Bax Bcl - XL Bcl -
 2 family dimerization E1B - 19K Bax dime -
 rization 7)
 E1B - 19K
 8), 15),
 ulum¹⁶⁾, 23)
 가
 , Bax
 , staurosporine Tumor Necrosis Factor -
 (TNF -) Bax가 mitochondria
 24)
 E1B - 19K가 Bax
 dimerization E1B - 19K가 staurosporine
 TNF - Bax mitochondria
 가
 Green fluo -
 rescent protein²⁴⁾(GFP) Bax E1B - 19K
 apoptosis
 confocal microscope
 in vivo E1B - 19K
 Bax dimerization

대상 및 방법

1. 대상 및 재료

Cos - 7 cell ATCC
 primer GIBCO BRL . pcDNA3
 mammalian expression vectors Invitrogen(Carlsbad,
 CA) , C3 - EGFP plasmid Clontech
 Laboratories Inc.(Palo Alto, CA) . Lipofe -
 ctamine Life Technologies(GIBCO BRL, Gaithe -
 rsburg, MD) , E1B - 19K adeno - virus
 type2 EcoR1 clonning site가
 Dr. Castelli JC(SNB, NINDS, NIH, MD, USA)
 (White Cipriani
²²), Bax Dr. Hsu YT
 Sigma Chemical Co.(St. Louis, MO)

2. 방 법

1) Plasmid 제작

E1B - 19K C3EGFP pcDNA - 3 vector EcoR1
 digestion gel electrophoresis

ligation C3 - EGFP - E1B - 19K pcDNA - 3
 E1B - 19K Bax PCR
 3' 5' EcoR1 site C3EGFP
 pcDNA - 3 vector EcoR1 digestion gel ele -
 ctrophoresis ligation C3 -
 EGFP - Bax pcDNA - 3 Bax
 2) Cell transfection
 Cos - 7 cell 4.3cm² chamber
 slide(Lab - Tek chambered coverglass system ; Na -
 lge Nunc Inc., Naperville, IL) 24
 plasmid Lipofectamine temporary tran -
 sfection . DNA transfection 0.5 µg
 , C3 - EGFP plasmid pcDNA - 3 tran -
 sfection 0.5 µg, 2.0 µg 1 : 4
 . Transfection Lipofectamine 3 µl 100
 µl Optimem solution , 100 µl Optimem solution
 DNA , solution 45
 , Optimem solution 1.3ml Cos -
 7 가 5 CO₂
 . Optimem solution Cos - 7
 10% FBS DMEM

3) Confocal microscopy

4.3cm² chamber slide Cos - 7 DNA
 transfection 16 24 confocal microscope
 . mitochondria mi -
 tochondria - specific dye(Mitotracker Red CMXRos ;
 Molecular Probes Inc., Eugene, OR) 20ng/m가
 20 confocal microscope(a model LSM
 410 confocal, Carl Zeiss, Thornwood, NY))
 . Chamber slide 35 37 air
 stream incubator . GFP
 580nm , mitotracker
 420nm . staurosporine
 가 0.5 µM , confocal mi -
 croscope 5 10 4 6

결 과

1. GFP-E1B-19k의 세포 내 위치

Cos - 7 monkey kidney epithelial cell
 GFP - E1B - 19K

mitochondria Mitotracker
 GFP E1B-19K
 mitochondria
 (Fig. 1 - A).
 E1B-19K가 apoptosis가
 staurosporine
 apoptosis E1B-
 19K apoptosis가 Bcl-XL
 (Fig. 1 - B).

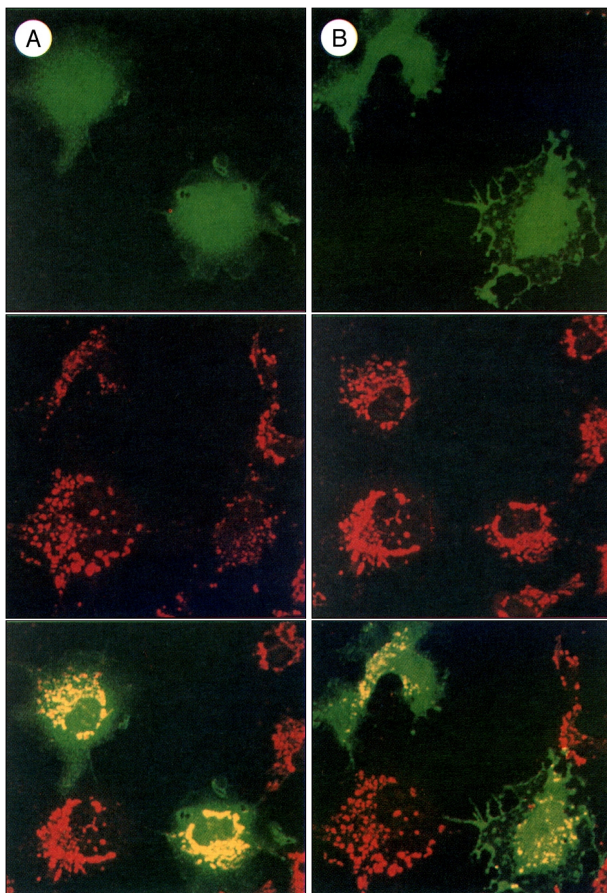


Fig. 1. The subcellular location of E1B-19K in vivo is diffuse in cytosol and nucleus in Cos-7 cells but is not changed to mitochondria with staurosporine. Cos-7 cells transiently transfected with GFP-E1B-19K and treated with Mitotracker Red CMXRos to stain mitochondria were examined by laser fluorescence confocal microscopy at 480nm wavelength for GFP(top) and at 560 nm for Mitotracker Red CMXRos(middle), and the two images overlaid(bottom). A : Green color of GFP-E1B-19K is diffuse in cytosol and nucleus and does not colocalize with the red color of mitochondria in cytoplasm of Cos-7 cells. B : Green color of GFP-E1B-19K is diffuse in cytosol and nucleus and does not colocalize with the red color of mitochondria in the cytoplasm of Cos-7 cells even when apoptosis is induced with staurosporine (0.5 μ M).

2. GFP-Bax의 세포 내 위치
 Bax , mitochondria,
 가 ,
 GFP
³⁴⁾ . GFP
 Bax
 (Fig. 2 - A).
 Staurosporine apoptosis가
 Bax E1B-19K Bcl-XL ¹²⁾ GFP -
 mi -

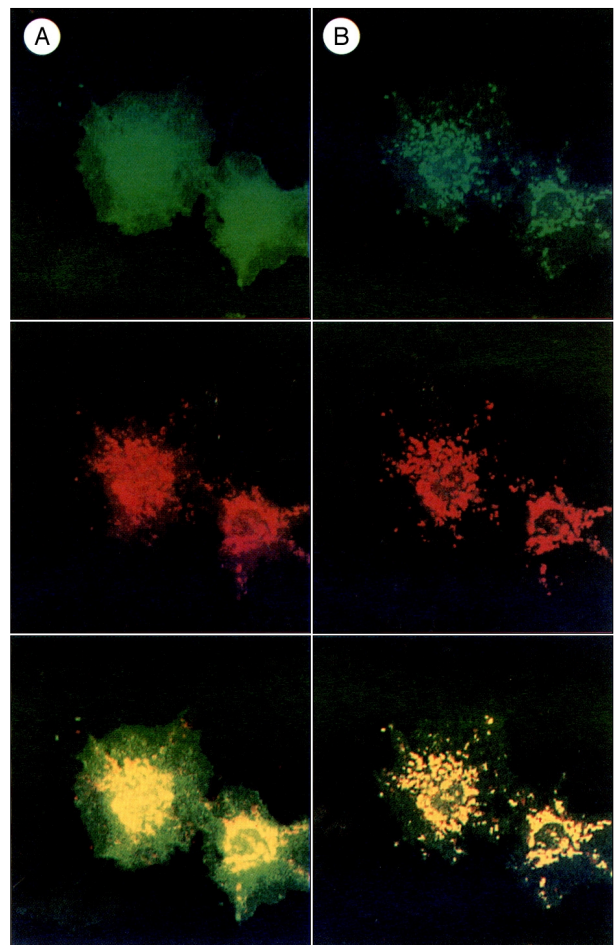


Fig. 2. The subcellular location of Bax in vivo is diffuse in cytosol and nucleus in Cos-7 cells and is moved to mitochondria with staurosporine. Cos-7 cells transiently transfected with GFP-Bax and treated with Mitotracker Red CMXRos to stain mitochondria were examined by laser fluorescence confocal microscopy at 480nm wavelength for GFP(top) and at 560nm for Mitotracker Red CMXRos(middle), and the two images overlaid (bottom). A : Green color of GFP-Bax is diffuse in cytosol and nucleus and only partially colocalize with the red color of mitochondria in the cytoplasm of Cos-7 cells. B : Green color of Bax is translocated to mitochondria and colocalized precisely with the red color of mitochondria in the cytoplasm of Cos-7 cells when apoptosis is induced with staurosporine(0.5 μ M).

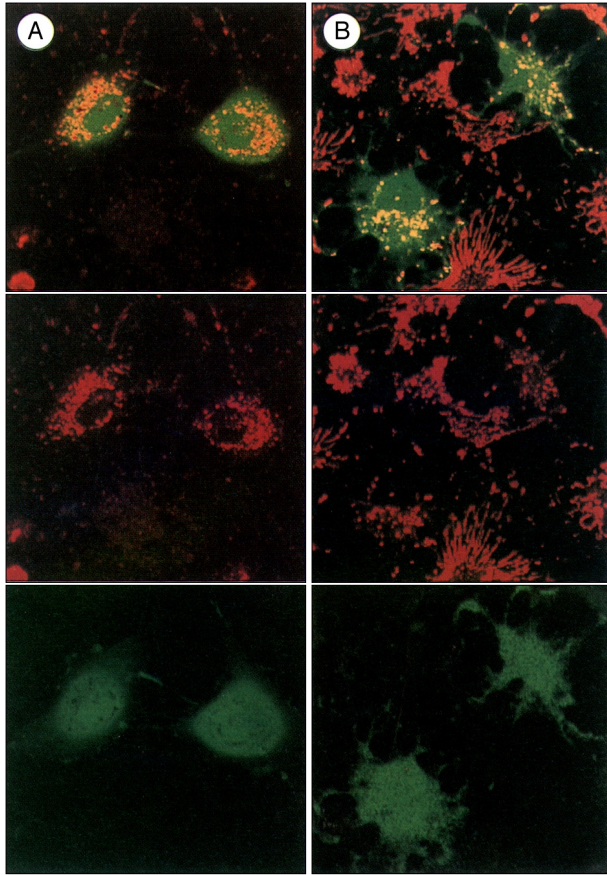


Fig. 3. The subcellular location of E1B-19K in vivo is still diffuse in cytosol and nucleus in Cos-7 cells and is not moved to mitochondria with staurosporine when cotransfected with Bax. Cos-7 cells transiently transfected with GFP-E1B-19K and pcDNA3-Bax and treated with Mitotracker Red CMXRos to stain mitochondria were examined by laser fluorescence confocal microscopy at 480nm wavelength for GFP (top) and at 560nm for Mitotracker Red CMXRos (middle), and the two images overlaid (bottom). A: Green color of GFP-E1b-19K is still diffusely cytosol and nucleus and generally does not colocalize with the red color of mitochondria in the cytoplasm of Cos-7 cells. B: Green color of E1B-19K is not moved to mitochondria and does not colocalized precisely with the red color of mitochondria in the cytoplasm of Cos-7 cells when apoptosis is induced with staurosporine (0.5 μM). GFP-E1B-19K does not translocates to the mitochondria when cotransfected with Bcl-XL even on apoptosis.

mitochondria (Fig. 2 - B). GFP-Bax가 mitochondria apoptosis staurosporine 1.5 2 Mitotracker mitochondria 가

3. pcDNA3-Bax와 동시에 transfection된 GFP-E1B-19K의 세포내 위치

Bax pcDNA3 , E1B - 19K C3 - EGFP vec -

tor transfection confocal microscopy GFP - E1B - 19K GFP - E1B - 19K transfection 가 , mitochondria Mitotracker (Fig. 3 - A). apoptosis가 Bax가 mitochondria pcDNA3 - Bax GFP - E1B - 19K transfection apoptosis GFP - E1B - 19K E1B - 19K가 Bax dimerization GFP - E1B - 19K Bax mitochondria 가 Cos - 7 monkey kidney epithelial cell pcDNA3 - Bax GFP - E1B - 19K transfection staurosporine 가 apoptosis E1B - 19K apoptosis가 (Fig. 3 - B).

고찰

Bcl - 2 family apoptosis 가 apoptosis , proapoptotic protein Bax가 , antiapoptotic protein Bcl - 2 Bcl - XL 5) Bax가 mitochondria 20) mitochondria cytochrome - C가 17) cas - pase 26) 13) apoptosis가 14) Bcl - 2 family dimerization 가 yeast functional assay immunoprecipitation 9)19) 가 anti - apoptotic Bax hetero - dimerization 25) Bax Bax homo - dimerization 가 mitochondria docking 6) cytochrome C가 17) 13) 가 1997 dimerization 가 Hsu 11) dimerization 1998 Hsu 10) dimerization detergent Bcl - 2 family homo - hetero - dimerization 가 Bcl - 2 family dimerization 가

E1B - 19K 가 Bax Bax chondria .
 Bax Bax homo - dimerization , Bax E1B - 19K dime -
 Bax mitochondria do - rization dimerization
 cking cytochrome C apoptosis GFP
 apoptosis 7)8) 가 가 가
 Bax homo - dimerization, Bax E1B -
 19K hetero - dimerization 가 , dimerization
 E1B - 19K Bax hetero - dimerization apoptosis 가
 dimerization 가
 가 E1B - 19K 가 E1B - 19K GFP
 가 E1B - 19K Bax dimerization
 가 GFP di -
 merization
 dimerization 가 () E1B - 19K Bax가 dim -
 western blotting, immunoprecipitation erization GFP
 in vitro 가 가 가
 가 . In vivo
 GFP E1B - 19K
 Bax confocal mi -
 croscopy dimerization GFP E1B - 19K cytosol nucleus
 , 가 apoptosis가 , Bax Bax
 Bcl - XL mitochondria rine apoptosis가 staurospo -
 apoptosis가 mitochondria 12) sta -
 urosporine apoptosis Bcl - XL pro - E1B - 19K cytoplasm nucleus
 survival 가 E1B - 19K mitochondria tion , Bax apoptosis가 dimeriza -
 가 . GFP apoptosis
 E1B - 19K . Bax Bax
 mitochondria . Staurosporine apoptosis가 442 - 721 5
 mitochondria . GFP : 031) 219 - 5230, : 031) 219 - 5237
 E1B - 19K E1B - 19K E - mail : ee80@madang.ajou.ac.kr
 Bax가 apoptosis가 dimerization
 . E1B - 19K trans -
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 2), Bax Bax
 가 apoptosis 가
 () E1B - 19K apoptosis
 Bax hetero - dimerization
 .
 GFP E1B - 19K
 apoptosis Bax , mito -

결 론

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