Nitric Oxide As a Mediator of Apoptosis

Mallika Somayajulu

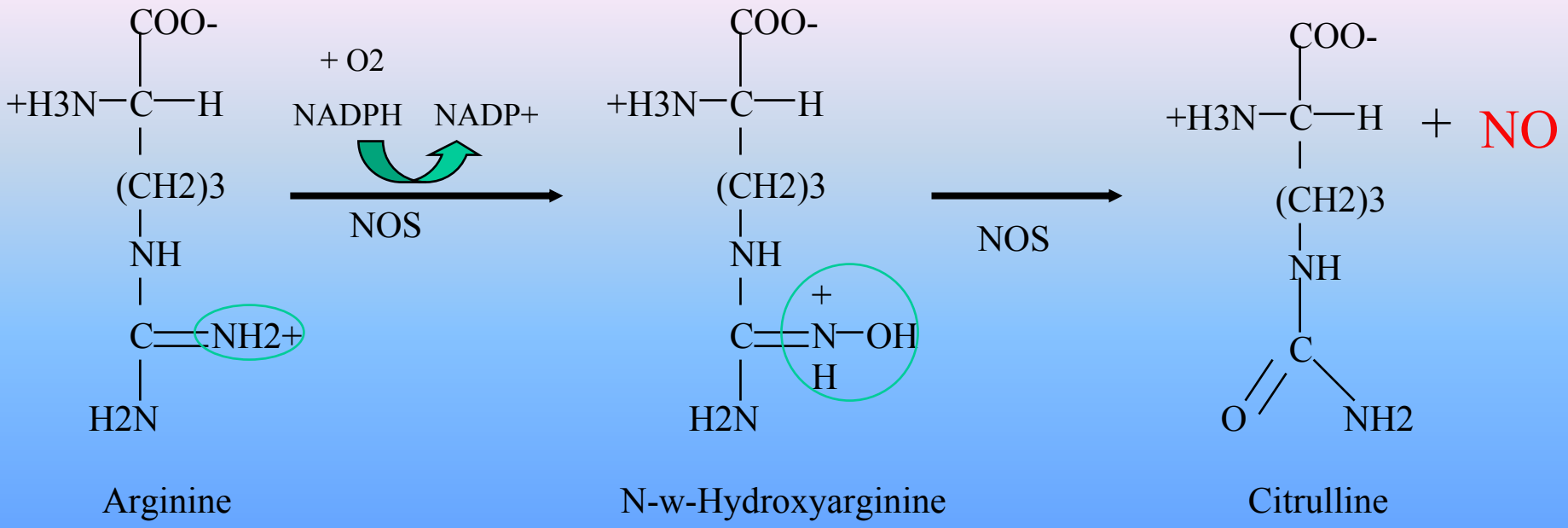
Introduction

Nitric Oxide:

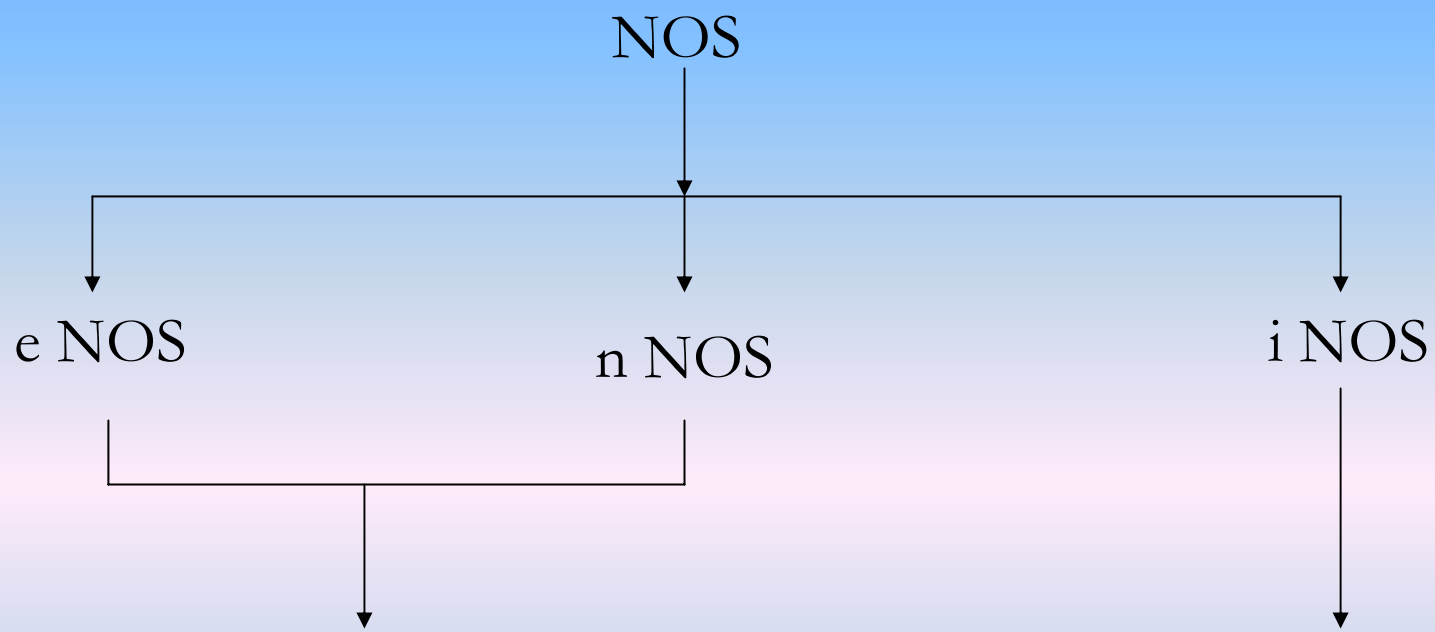
- A diatomic free radical consisting of one atom of nitrogen and one atom of oxygen.
- Highly reactive
- Very small so can easily pass between cell membranes.

Synthesis of Nitric Oxide

- Nitric oxide is synthesized from L-arginine.
- This reaction is catalyzed by nitric oxide synthase.



Arginine N-w-Hydroxyarginine Citrulline



- Activated by increased Ca^{+2}.

- Seen in macrophages after stimulation of inflammatory/immune reaction.

Effects of NO

Direct

-Is because of NO

•Metal complexes

-Can activate and inactivate many proteins

•Lipid radicals

Indirect

-Is due to formation of N_2O_3

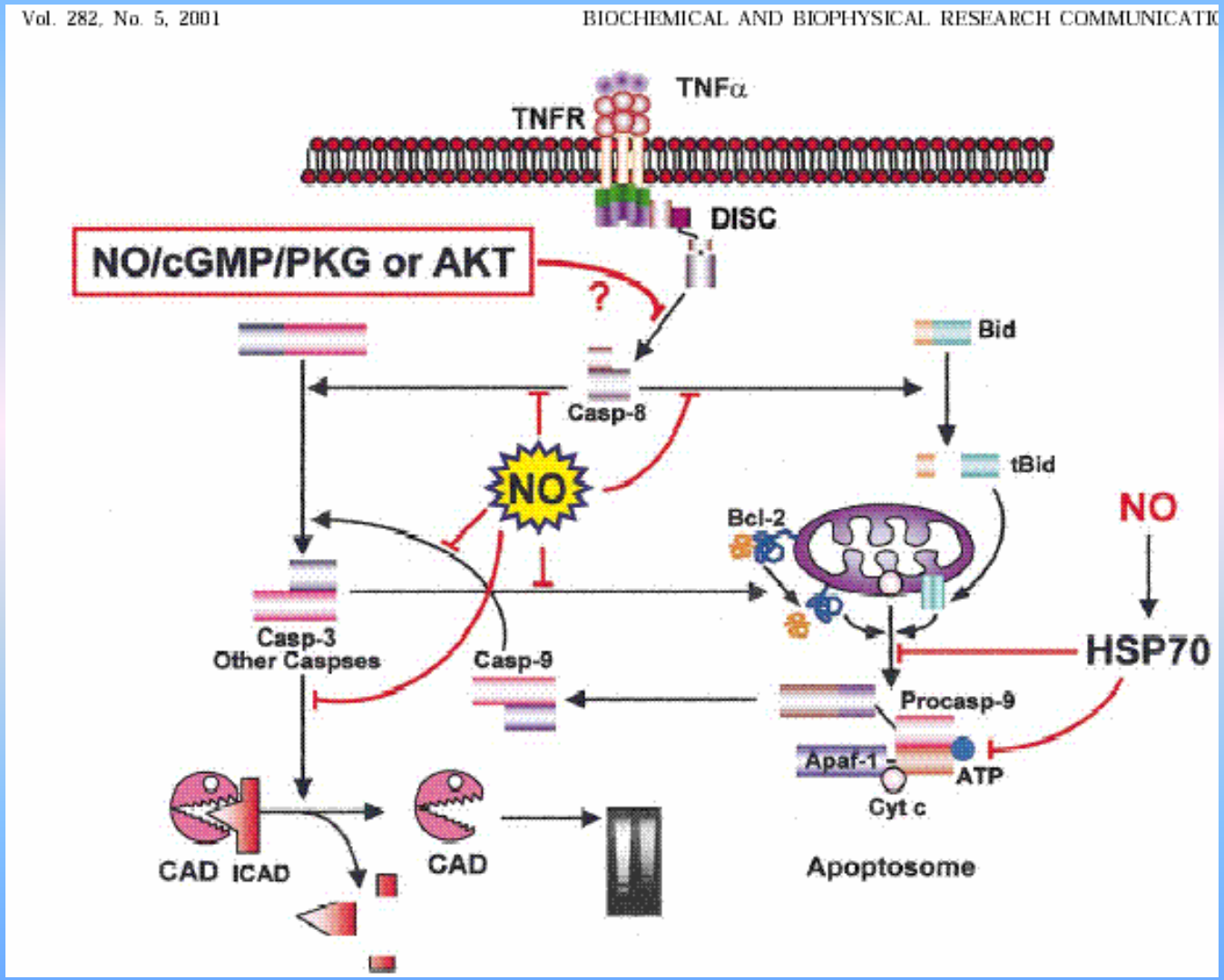
And ONOO-

•Nitrosation

•DNA strand breaks

•Nitration

NO as an anti-apoptotic agent



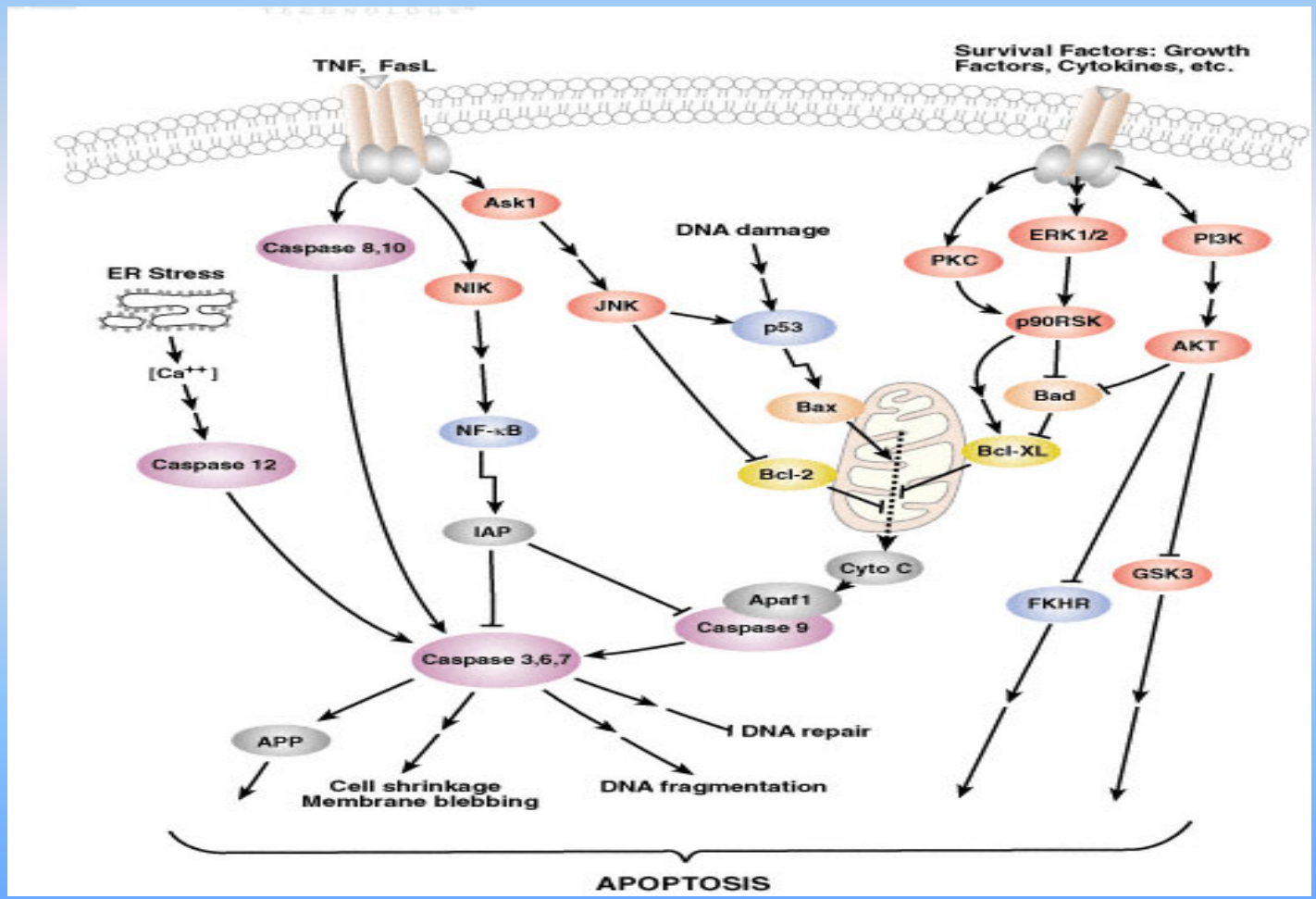
Apoptosis

- Programmed cell death.
- A physiological process in response to a specific stimuli.
- A cellular process regulated extrinsically and intrinsically.
- An active process in which specific genes are involved.
- May occur through specific signaling pathways.

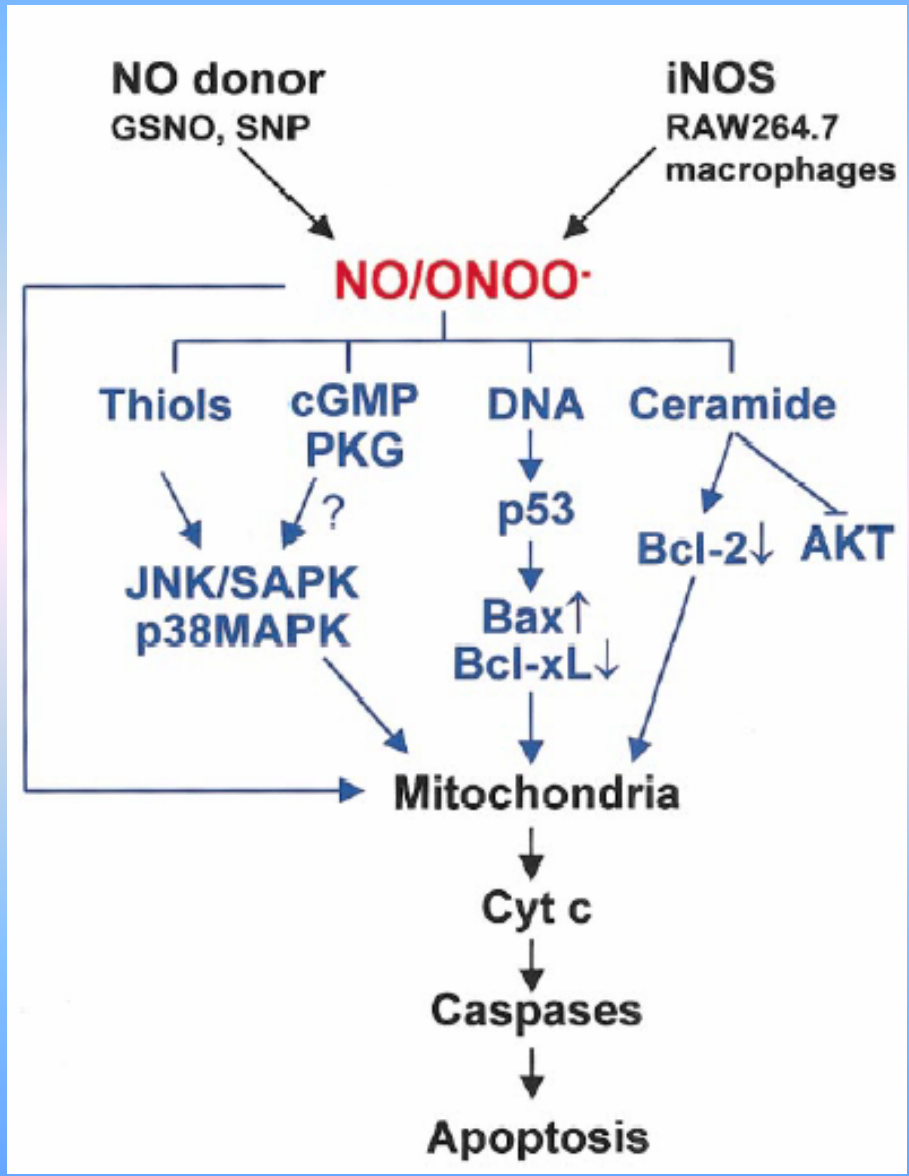
Physiological Significance

- Excessive cell death causes neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.
- Inhibition of cell death can also lead to hyperproliferative disease such as cancer.

Apoptotic Pathway



NO Signaling in Apoptosis



(Biochemical and biophysical Research Communications 2001)

Nitric oxide induced apoptosis-

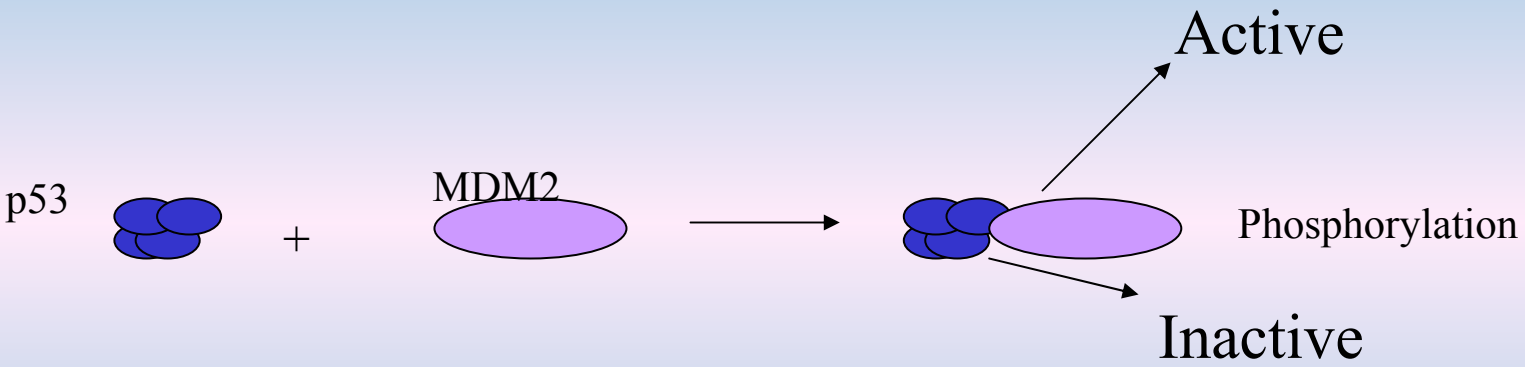
Is it p53 dependent?

NO Induces Apoptosis in Murine Primary Neural Cells by a p53 Dependent Pathway

Kaji T.,Kaieda I., HisatsuneT., Kaminogawa S., Nitric Oxide(2002)

- Neural cells from p53 positive and p53 knock out mice were treated with SIN-1 and apoptosis was studied.
- Mechanism for p53 accumulation by SIN-1 was analyzed.
- Western blot revealed that p53 accumulation didn't require p53 phosphorylation.
- P53 accumulation by ras-MAPK-p19 pathway.

P53



- MDM2 binds to N-terminal region of p53 ⟶ inhibits p53 mediated transcription

DNA damage and p53

- DNA damage activates the DNA dependent protein kinases.
- Phosphorylation of Ser (15) in the N terminal.
- Mdm2 dissociates from p53 , making it active.
- P53 induces the transcription of Bax and ultimately leads to apoptosis.

TABLE I

Evaluation of Apoptosis in Mouse Neural Primary Cultures

	TUNEL-positive cells			DNA fragmentation
Treatment	0 h	12 h	24 h	8 h
Control	12.7 ± 4.04			5.54 ± 1.39
UV (15 J/m^2)		50.2 ± 5.35	68.7 ± 2.25	25.74 ± 5.53
SIN-1 (200 μM)		51.9 ± 1.13	61.1 ± 0.74	24.31 ± 5.91
SIN-1 (50 μM × 4)			50.1 ± 3.38	

Note. TUNEL-positive cells and the percentage of DNA fragmentation were calculated as described under Materials and Methods. Data are the means ±S.D. of three independent experiments. The unit is %.

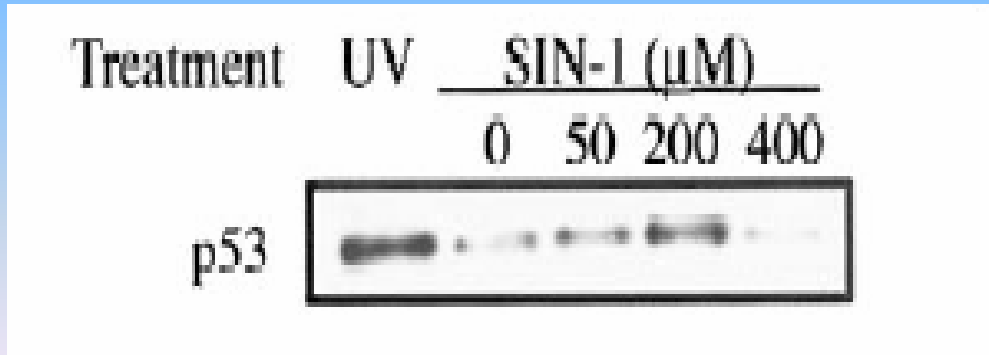
TABLE II

TUNEL-Positive Cells among $p53^{+/-}$ and $p53^{-/-}$ Cells

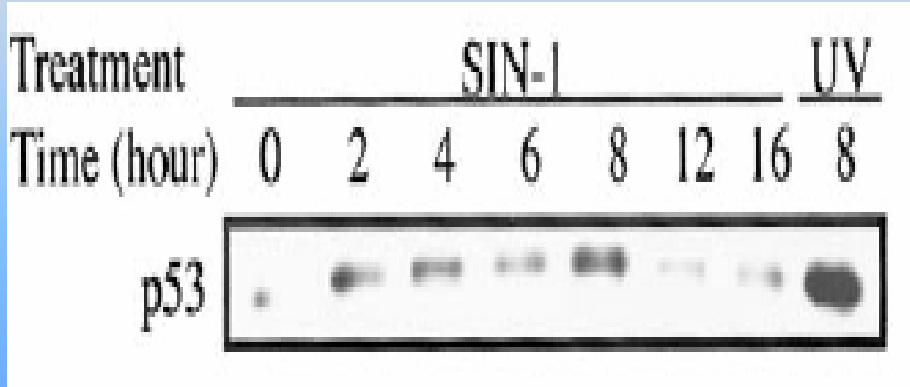
	Conc. of SIN-1		
	Untreated	200 μM	400 μM
$p53^{+/-}$ cells	8.57 ± 2.86	92.6 ± 5.14	98.2 ± 1.34
$p53^{-/-}$ cells	10.7 ± 3.57	30.7 ± 8.42	97.6 ± 0.88

Note. After SIN-1 treatment for 48 h, the $p53^{+/-}$ and $p53^{-/-}$ cells were fixed and the TUNEL assay was performed. Data are the means ±S.D. of four independent experiments. The unit is %; Conc. = Concentration.

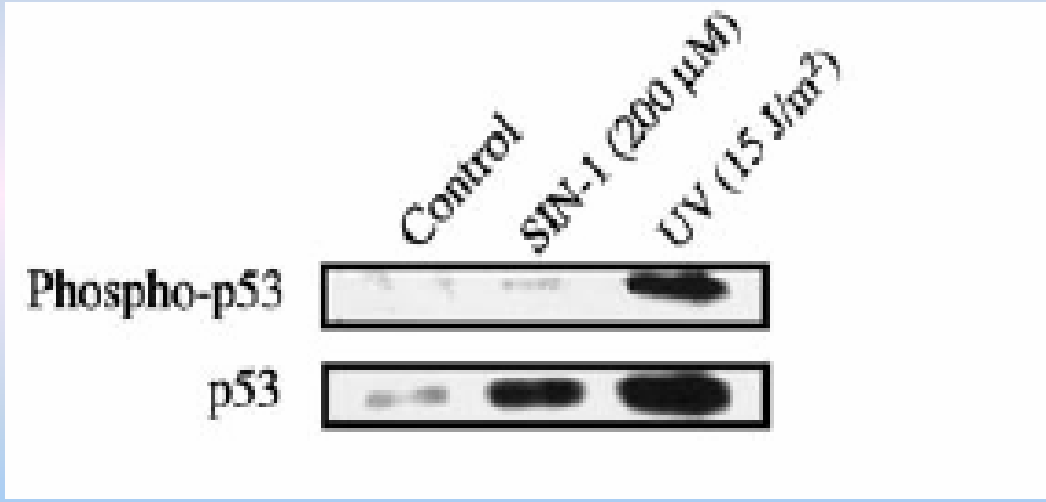
Dose dependent increase in p53 levels



Time dependent increase in p53 levels



- SIN-1 induced p53 accumulation is not through DNA damage.

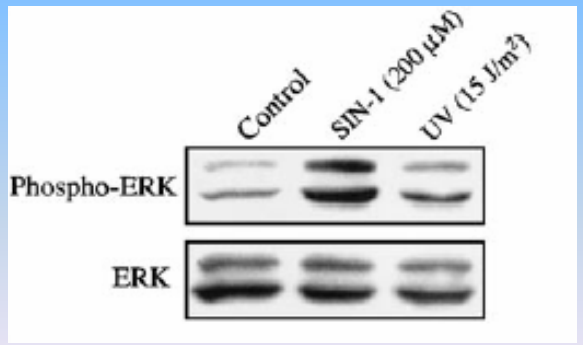


The Postulated Existence of Another Pathway

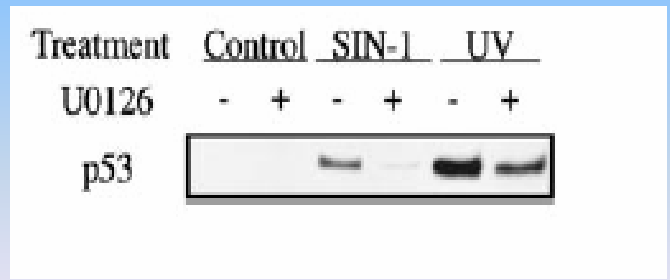
Based on the reports:

- NO stimulates p21 ras and MAPK activity.(1)
- p21 ras serves as a signal target of reactive free radicals.(2)
- p21 ras induces p53 accumulation via p19.(3)
- p19 functions as a bridge between p21 ras and p53 accumulation.(4)

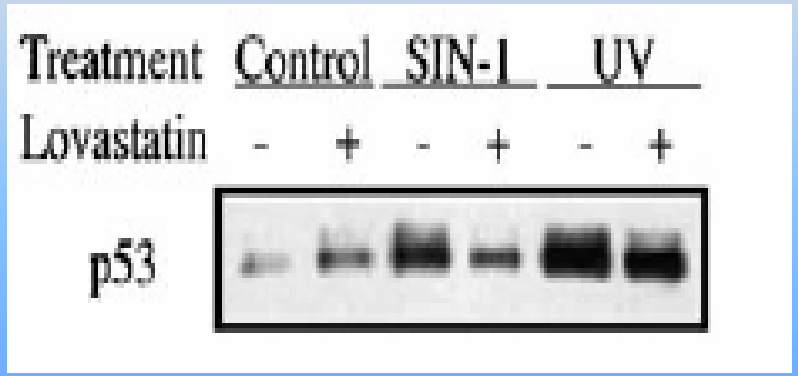
ERK activation



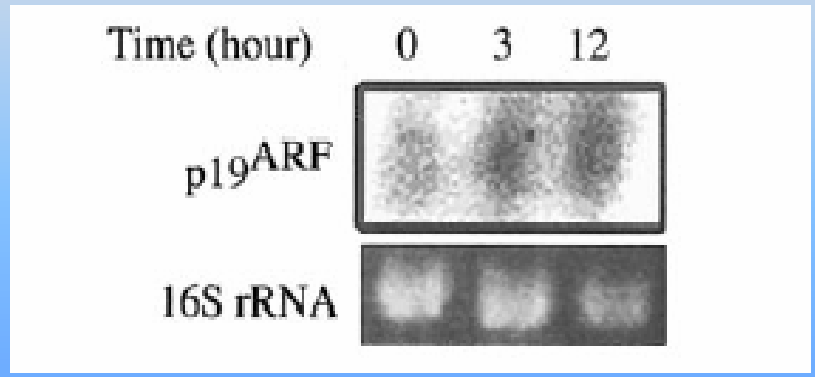
U0126 and p53



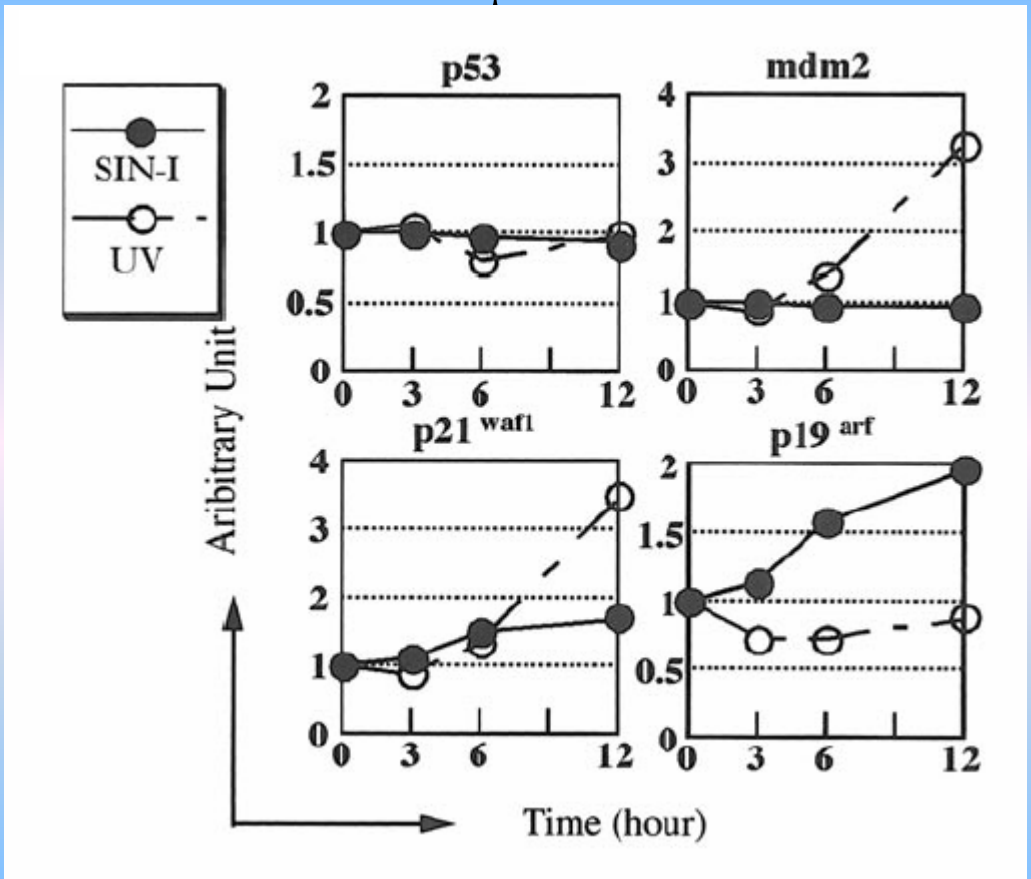
Lovastatin and p53



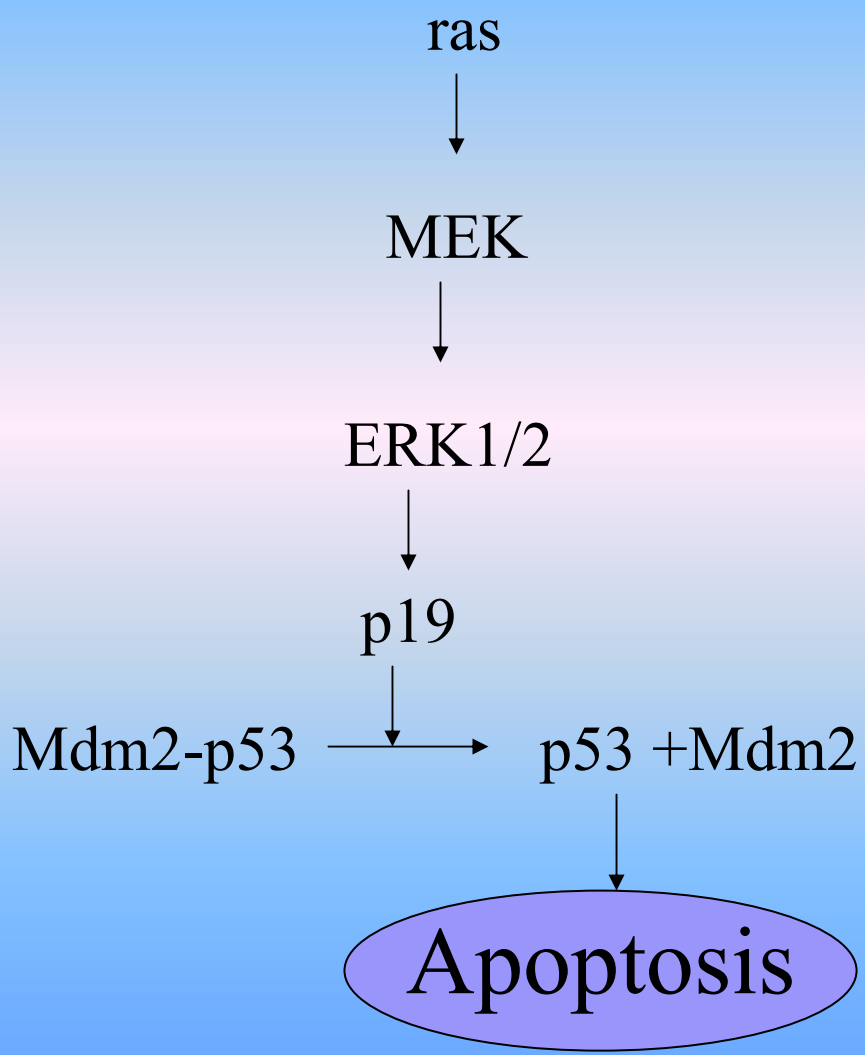
Northern Blotting



Levels of expression of p53 – related mRNA after treatment with 200μM SIN-1



PATHWAY



Apoptosis Occurs in p53 Deficient MG5 Microglial Cells by NO Through ER Stress

Kawahara K., Oyadomari S. , Gotoh T, Koshsaka S., Nakayama H., Mori M., FEBS (2001)135-139

ER Mediated Stress

- NO inhibits Ca-ATPase activity of ER by tyrosine Nitration .
- Activation of ryanodine receptor Ca+2 channel by nitrosylation of cysteine residues.
- Cells overcome ER stress by unfolding the proteins in the ER lumen.
- Involves the up regulation of different ER chaperones like Bip, Grp78 and CHOP.

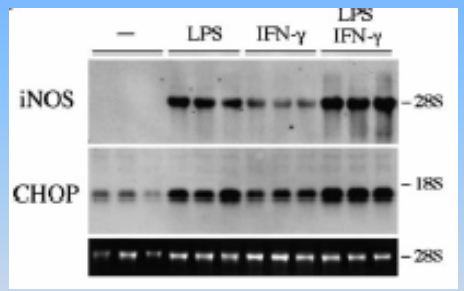
CHOP

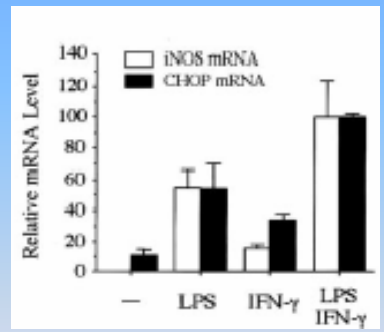
- C/EBP Homologous Protein.
- Transcription factor.
- Belongs to the family of Leucine zipper proteins.
- Have extensive amino acid homology with the DNA binding domain of C/EBP.
- Expressed at undetectable levels in growing mammalian cells.
- Considerably high under stress and treatment with genotoxic agents.

Experiment

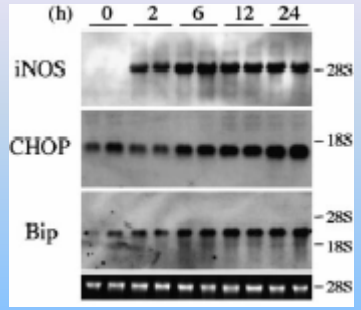
- Murine microglial cells were exposed to LPS(1μg/ml) and INF-γ (100U/ml) and mRNA and protein for iNOS and CHOP were induced and apoptosis occurred.
- CHOP and Bip mRNAs as well as proteins were expressed when cells were treated with SIN-1 and SNAP.

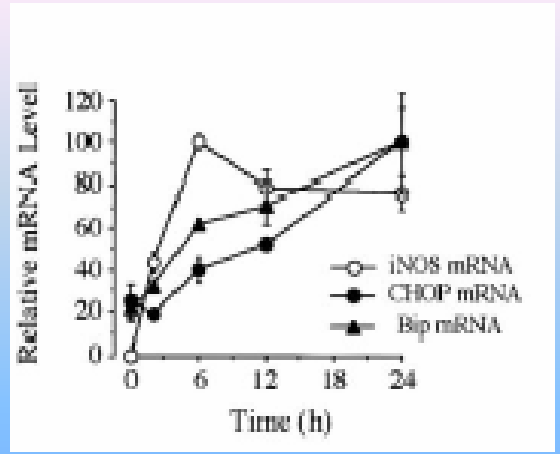
Induction of CHOP and iNOS mRNA by LPS/INF-γ



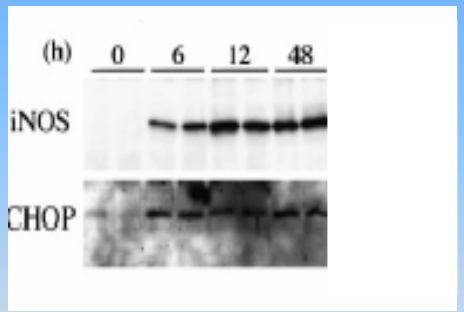


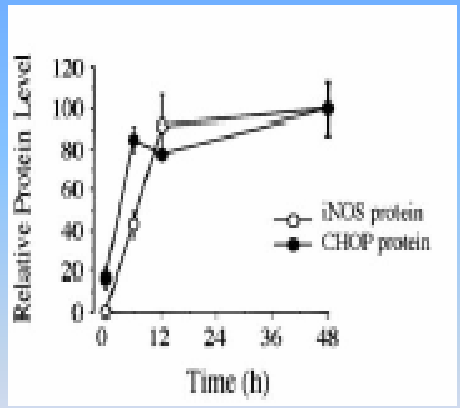
Induction of CHOP, iNOS and Bip mRNA by LPS/INF-γ



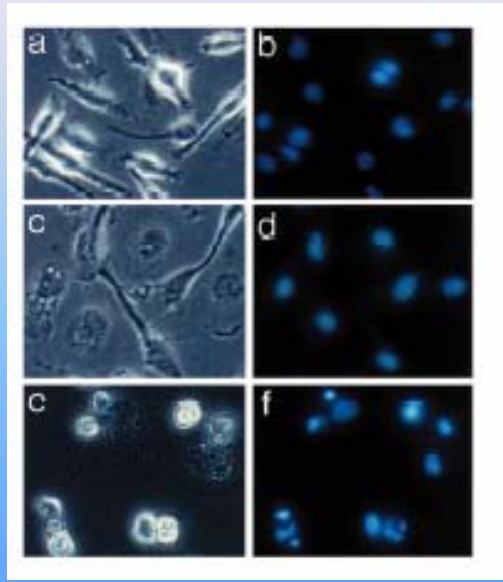


Induction of iNOS and CHOP Protein by LPS/INF-γ





Morphological changes induced by LPS/INF- γ

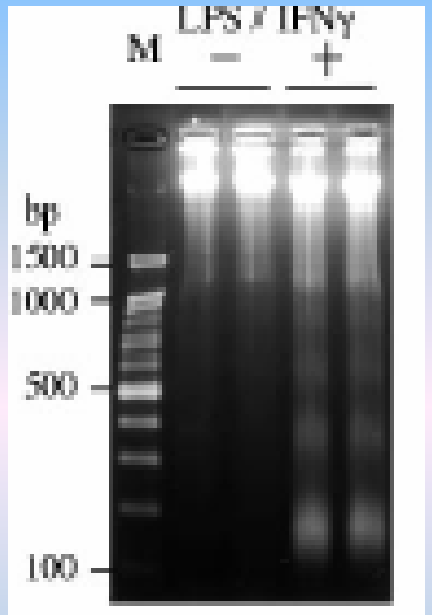


0 hours

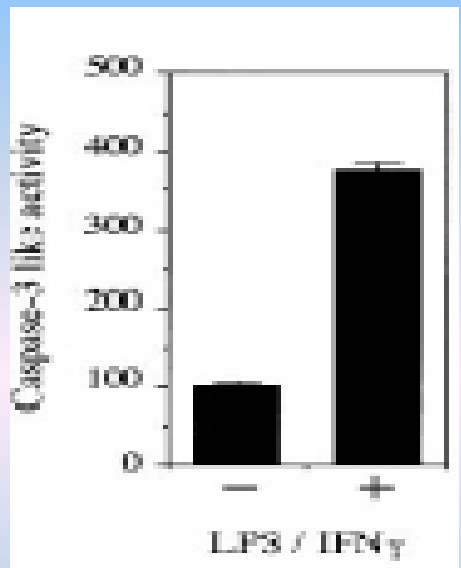
24 hours

48 hours

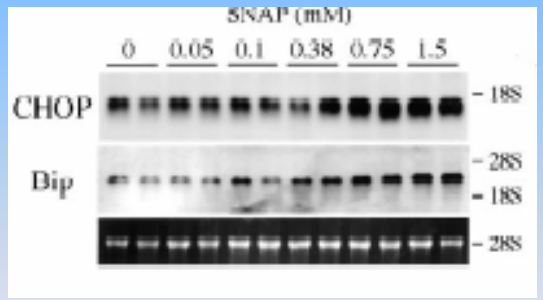
DNA Fragmentation

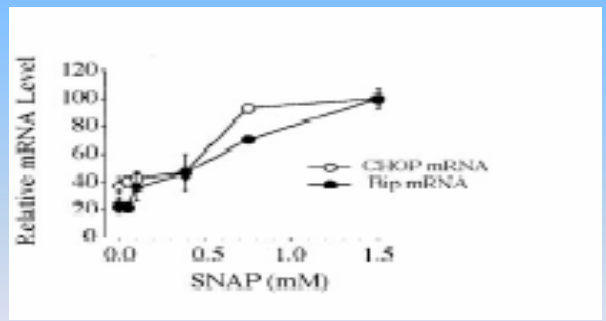


Caspase–3 like activity

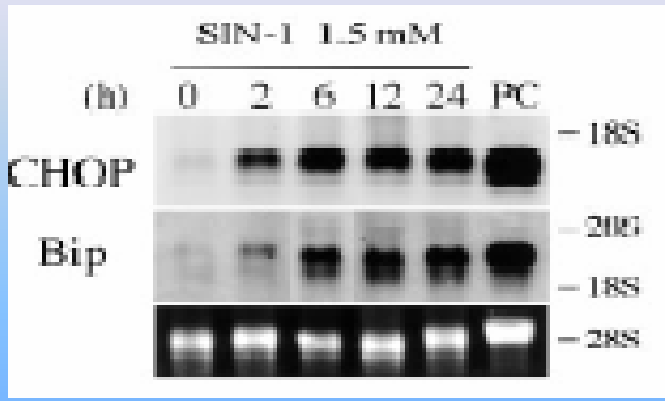


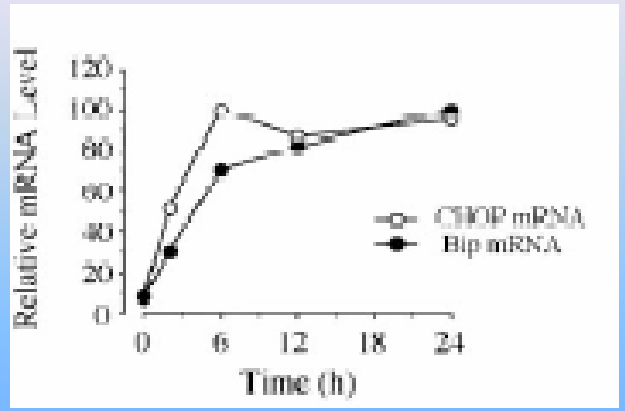
Induction of CHOP and Bip by SNAP



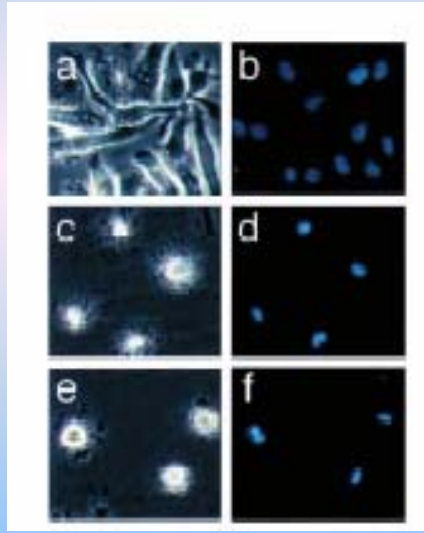


Induction of CHOP and Bip by SIN-1





Morphological changes

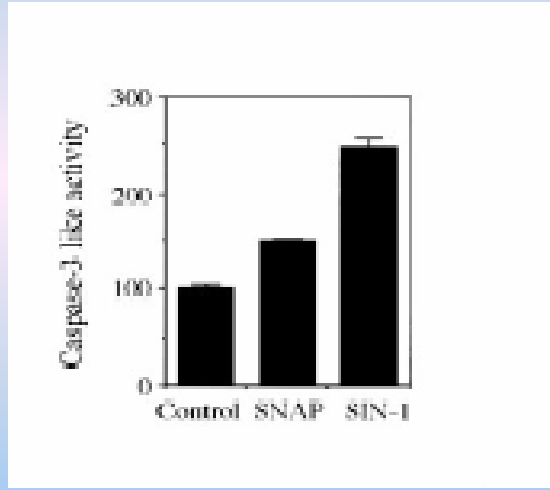


0 hours

24 hours

48 hours

Caspase-3 like activity



- Several genes on which CHOP could act downstream have been identified down stream of CHOP.
- But none is involved in programmed cell death.
- CHOP expression down regulates Bcl-2 expression, depletion of cellular glutathione, increases production of ROS.

(Mc Cullough et al, (2001)Mol.Cell.Biol.21, 1249-1259)

- Precise apoptotic cascade downstream has not been clarified.

Conclusions

- NO and peroxy nitrite induce apoptosis in different types of neural cells.
- Basis for new therapeutic intervention in various pathological states of the brain where NO plays an important role in development of cell injury.

References:

1) Chung ,H.T. et al (2001)No as a bioregulator of apoptosis. Biochem. Biophys.Res.Comm 282, 1075-1079.

2) Palmero,I.Pantoja,C.,and Serrano,M. (1998).p19ARFlinks the tumour suppressor p53 to ras.Nature 395, 125-126

3) Lander,H.M.et al(1995)p21 ras as a common signaling target of reactive free radicals and cellular redox stress.J.Biol.Chem 270,21195-21198

4) Sherr,C.J.,and Weber,J.D.(2000).The ARF/p53 pathway.Curr.Opin.Genet.Dev.10,94-99.

5) Ries,S.,Biederer,C.,Woods,D.,Shifman,O.Shirasawa,S.,Sasazuki,T.,McMahon,M., Oren,M., and McCormick,F.(2000).Opposing effects of ras on p53 transcriptional activation of mdm2 and introduction of p19 ARF.Cell 103,321-330.

6) Matsumoto,M.Minami,M.,Takeda,K., Sakao,Y.,Akira,S.,(1996)Ectopic expression of Chop induces apoptosis in M1 leukemia cells.FEBS Letters 395, 123-147