



Molecular Mechanisms of Sulfur Mustard

Vesicant-Induced Cell Death:

Early and late cell responses

**Peng Zhang¹, Michelle Yueqin Chen¹,
Diana Caridha¹, William J. Smith² &
Peter K. Chiang¹**

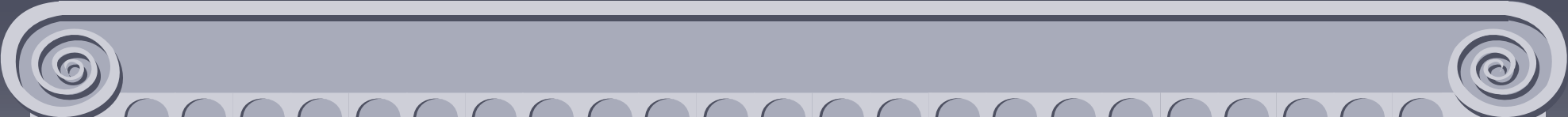
¹Walter Reed Army Institute of Research,

²United States Army Medical Research
Institute of Chemical Defense

Report Documentation Page				Form Approved OMB No. 0704-0188	
Public reporting burden for the collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to a penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number.					
1. REPORT DATE 01 OCT 2005		2. REPORT TYPE N/A		3. DATES COVERED -	
4. TITLE AND SUBTITLE Molecular Mechanisms of Sulfur Mustard Vesicant-Induced Cell Death: Early and late cell responses				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S)				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) Walter Reed Army Institute of Research				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release, distribution unlimited					
13. SUPPLEMENTARY NOTES See also ADM001851, Proceedings of the 2003 Joint Service Scientific Conference on Chemical & Biological Defense Research, 17-20 November 2003. , The original document contains color images.					
14. ABSTRACT					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 24	19a. NAME OF RESPONSIBLE PERSON
a. REPORT unclassified	b. ABSTRACT unclassified	c. THIS PAGE unclassified			

A decorative frame surrounding the text. It features two columns on the left and right sides, each topped with a spiral finial. A horizontal bar at the top contains a series of small, evenly spaced dots.

Introduction



Sulphur mustard reacts with a wide range of biological molecules, including proteins and nucleic acids. It possesses mutagenic, carcinogenic, cytotoxic, vesicating effects, and results in cell death.

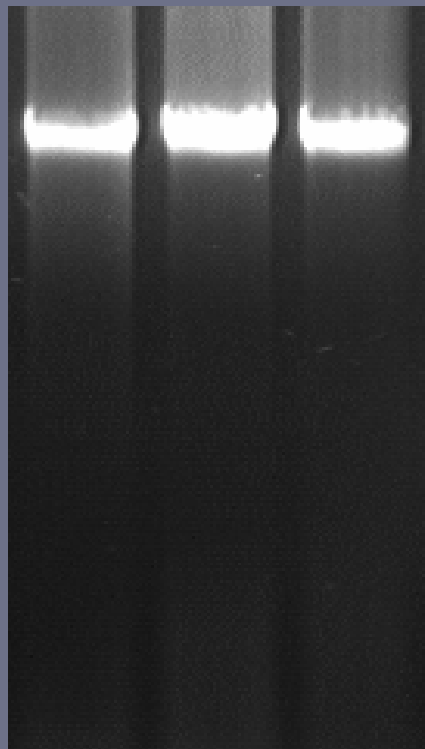
However, the biomedical mechanism of cell death induced by Sulphur mustard is not completely understood. To reveal this mechanism, we examined the specific genes involved in the regulation of cell survival and death pathway.



Experimental Results

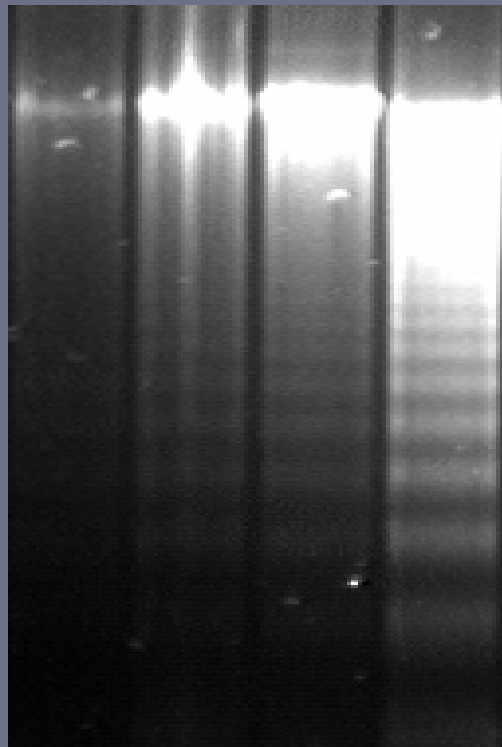
CEES Induced-Apoptosis

0 30 90 (min)

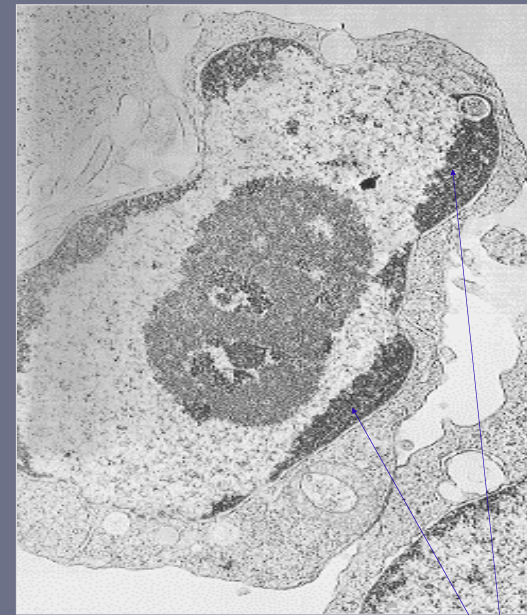


Early Stage

0 6 12 24 (h)

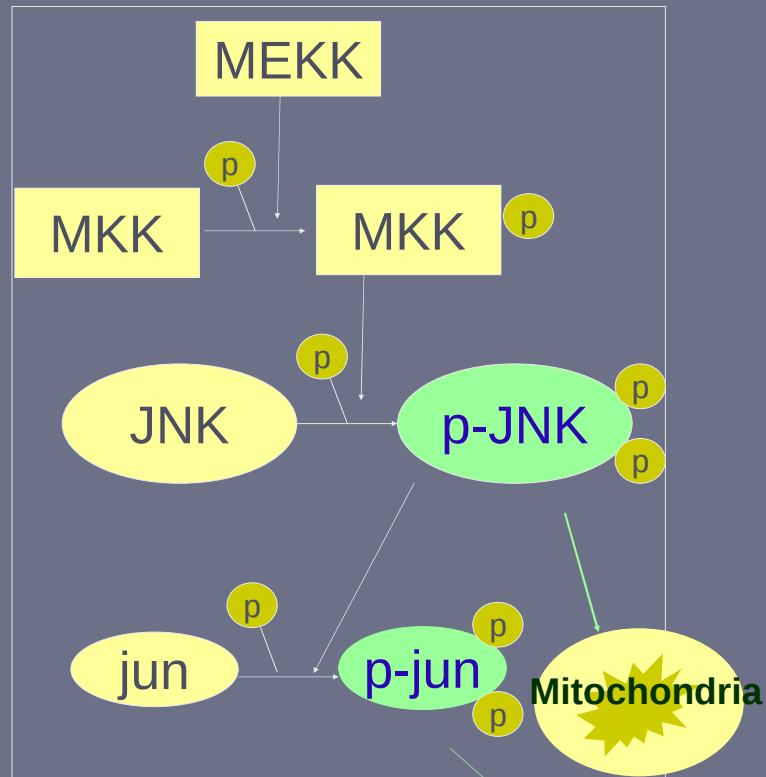


Late Stage

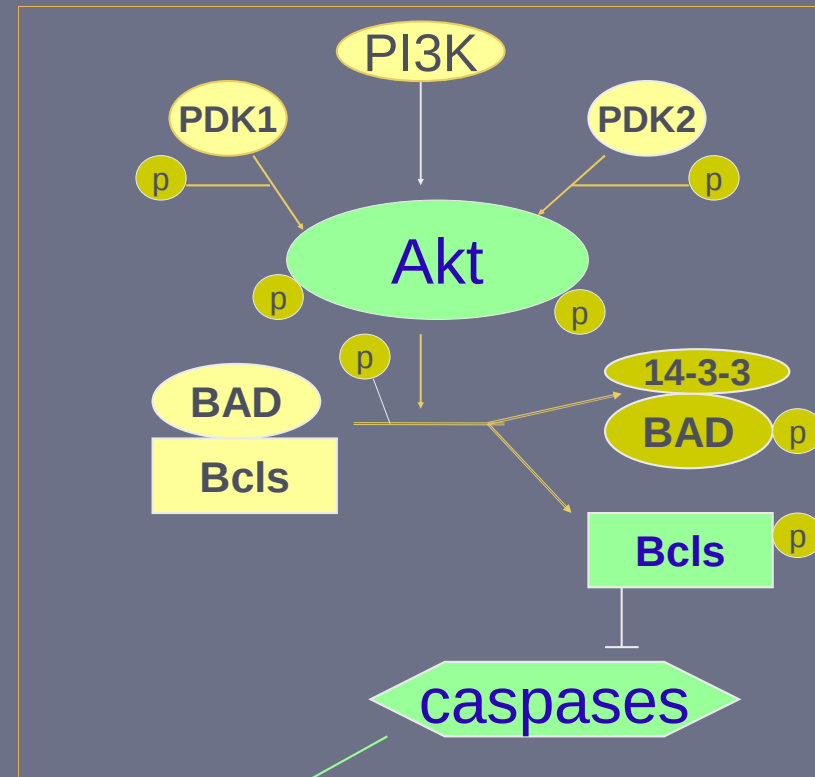


Perinuclear
margination
Of chromatin

Most Important Death Signal Transduction Pathway

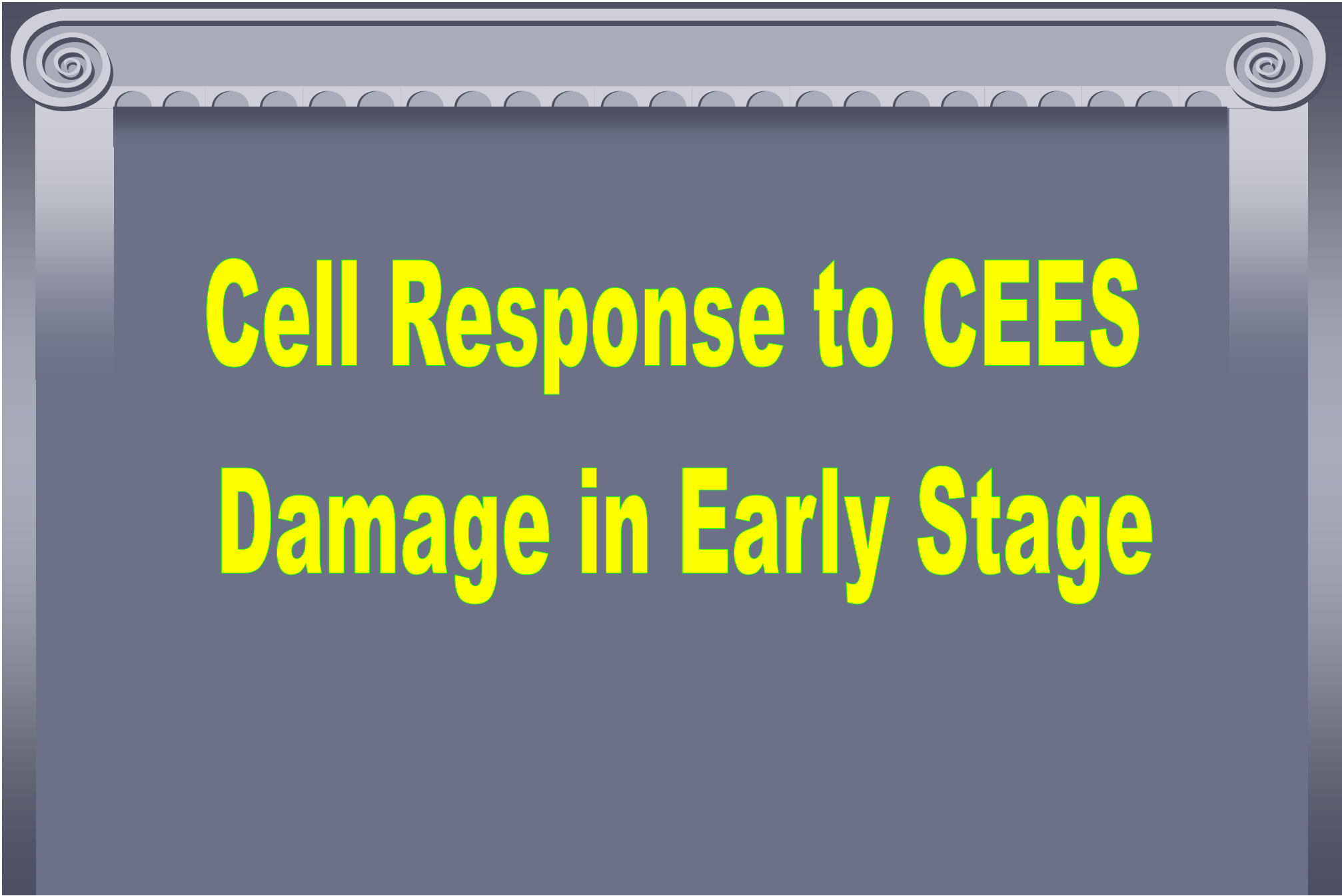


Early Stage



Late Stage

Apoptosis 



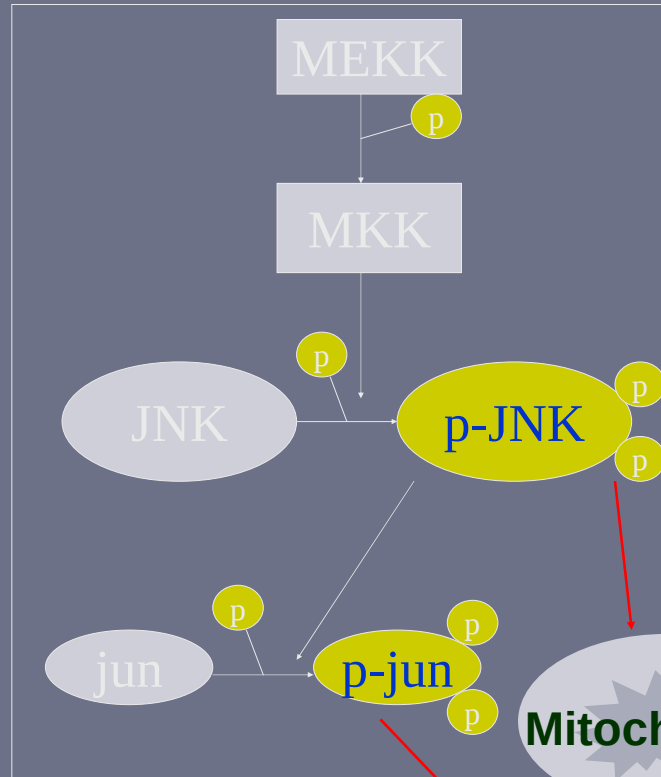
Cell Response to CEES

Damage in Early Stage

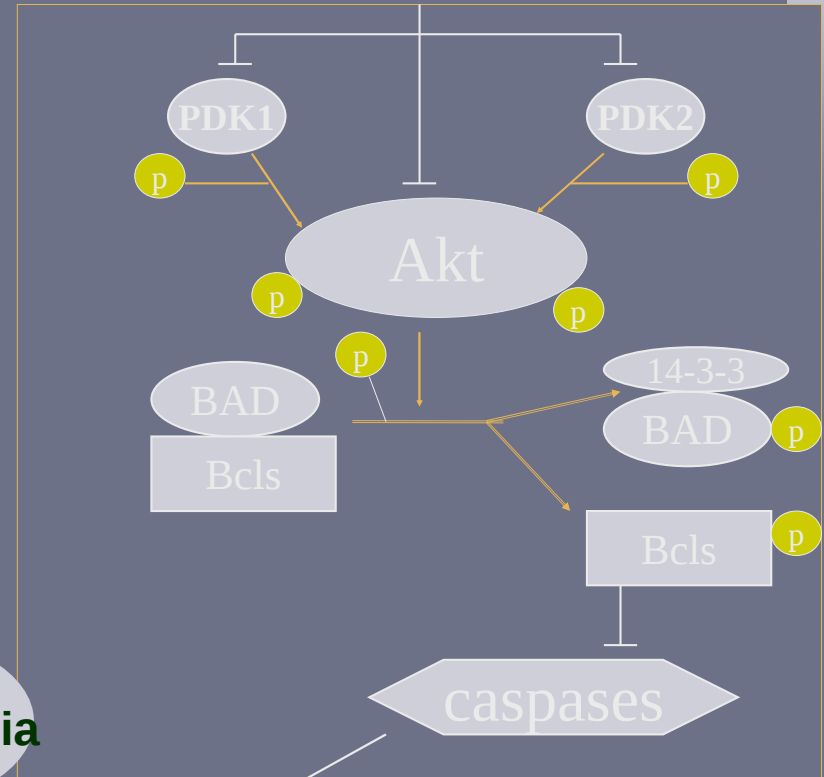
CEES

Attack

Cells



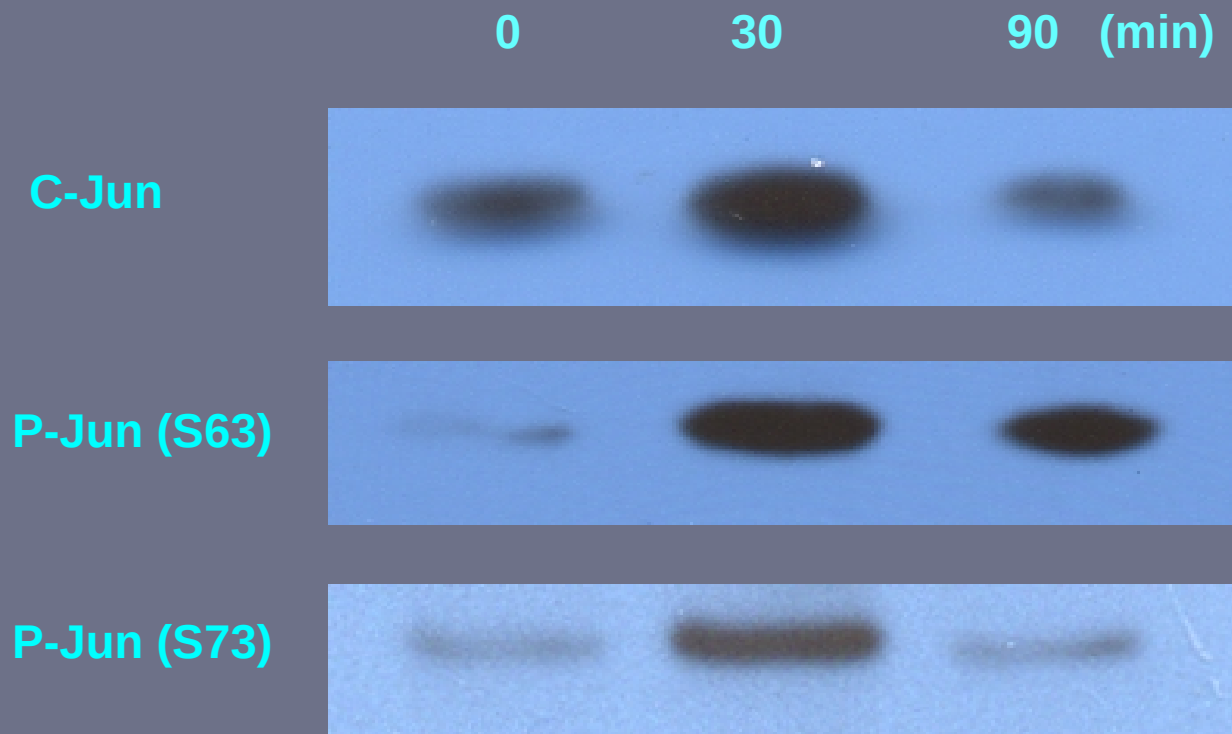
Early Stage



Late Stage

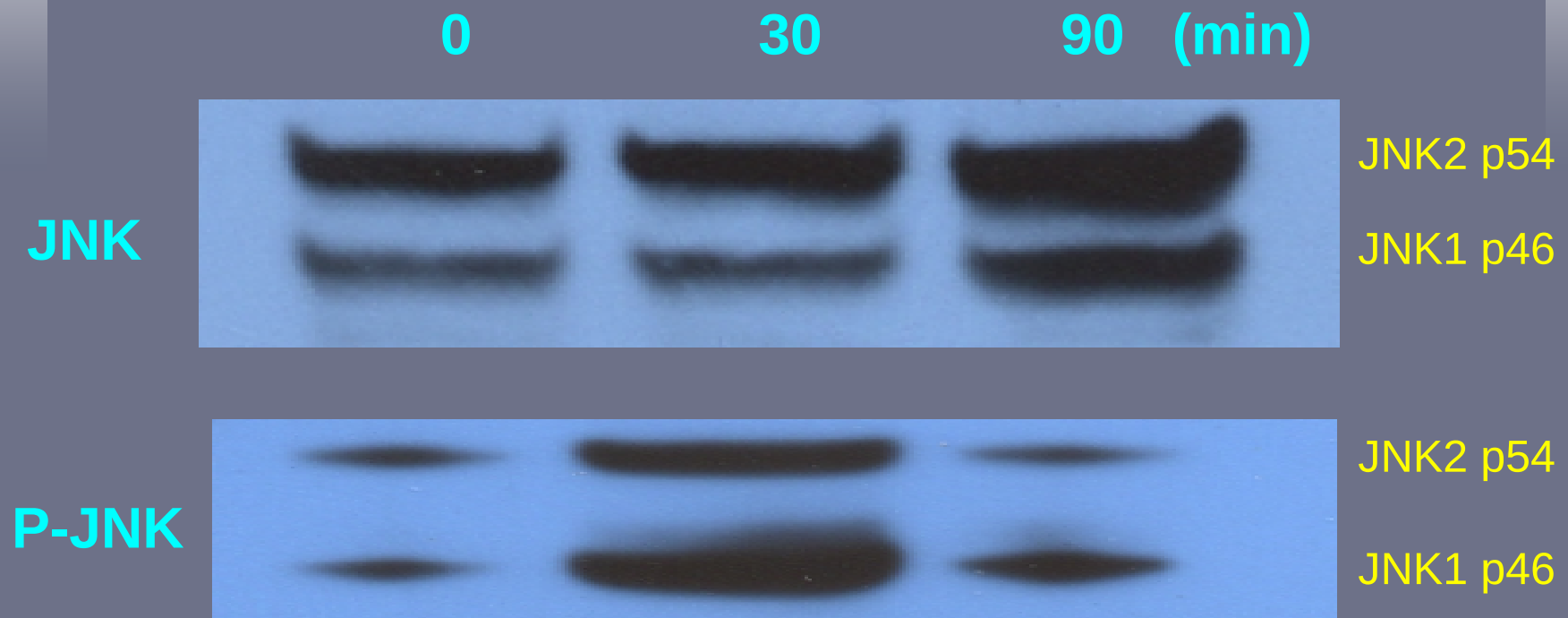
Apoptosis

CEES (200 μ M) Induced c-Jun and phosphorylation of Jun



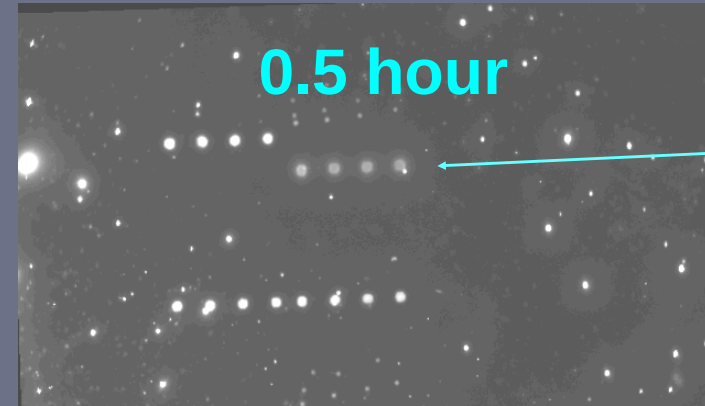
(Western Blotting)

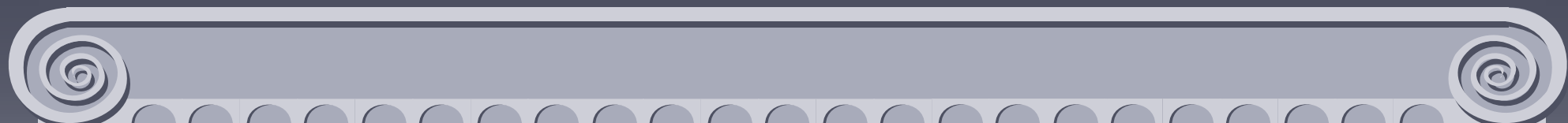
CEES (200 μ M) Induced JNK and Phosphorylation of JNK



(Western Blotting)

Cytokines Induced in Early Stage of CEES (200 μ M) Damaged in Jurkat cells

[illegible]

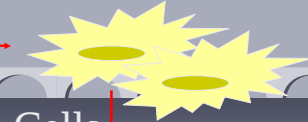


Cells Response to CEES

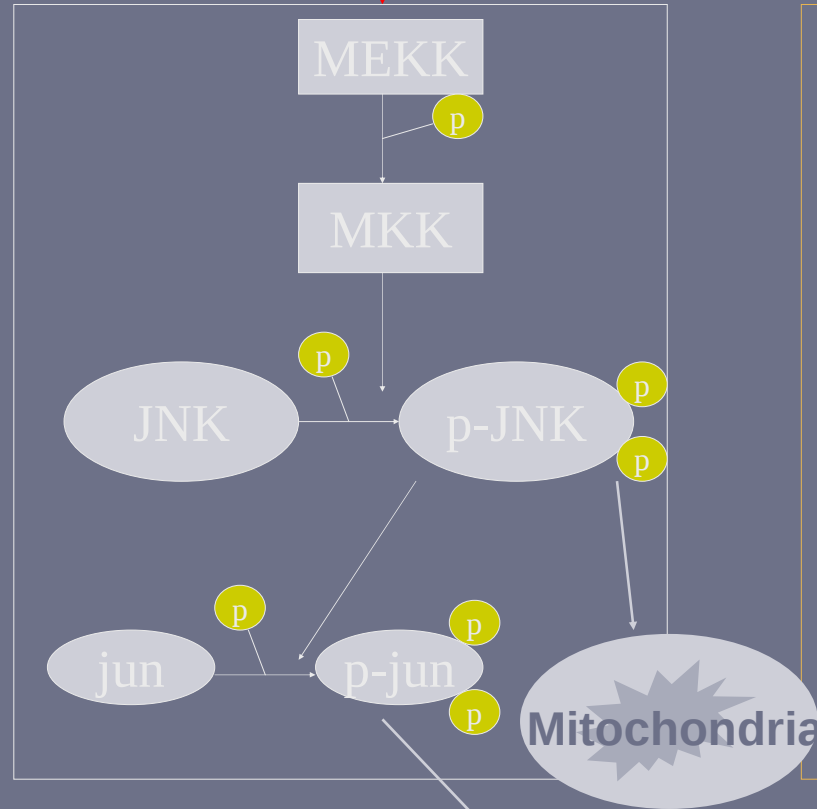
Damage in Late Stage

CEES

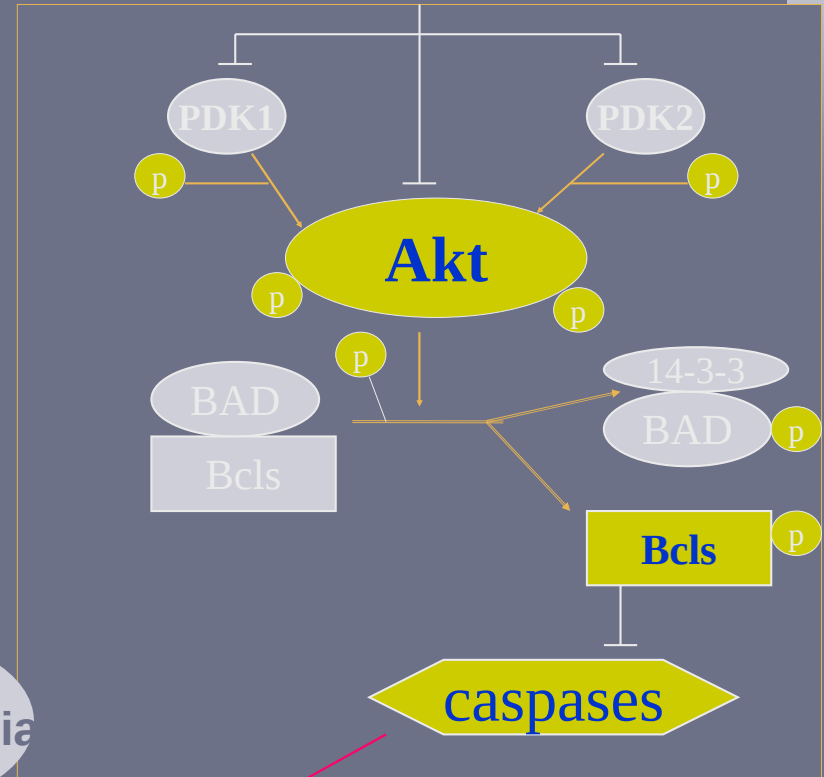
Attack



Cells



Early Stage

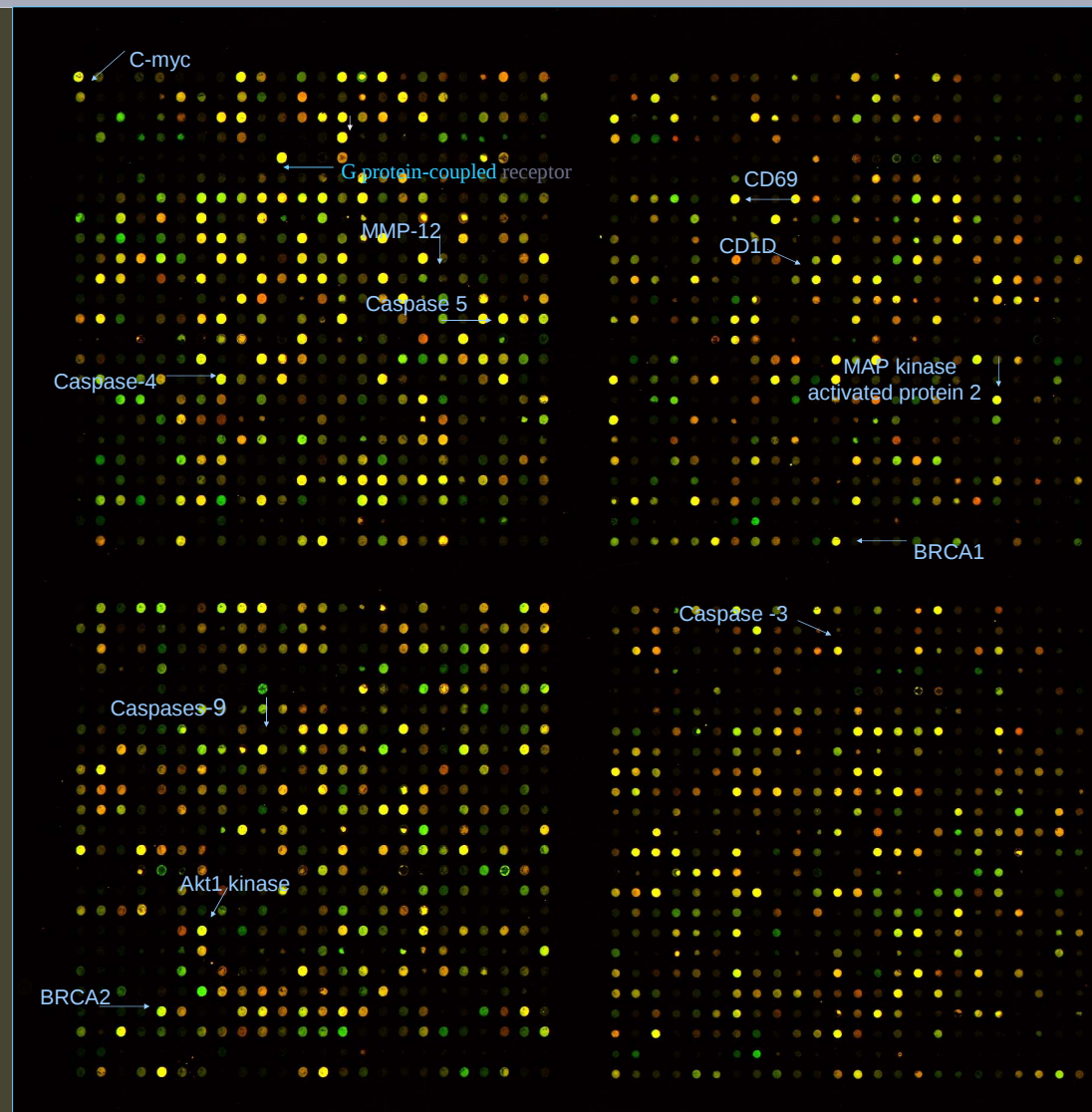


Late Stage

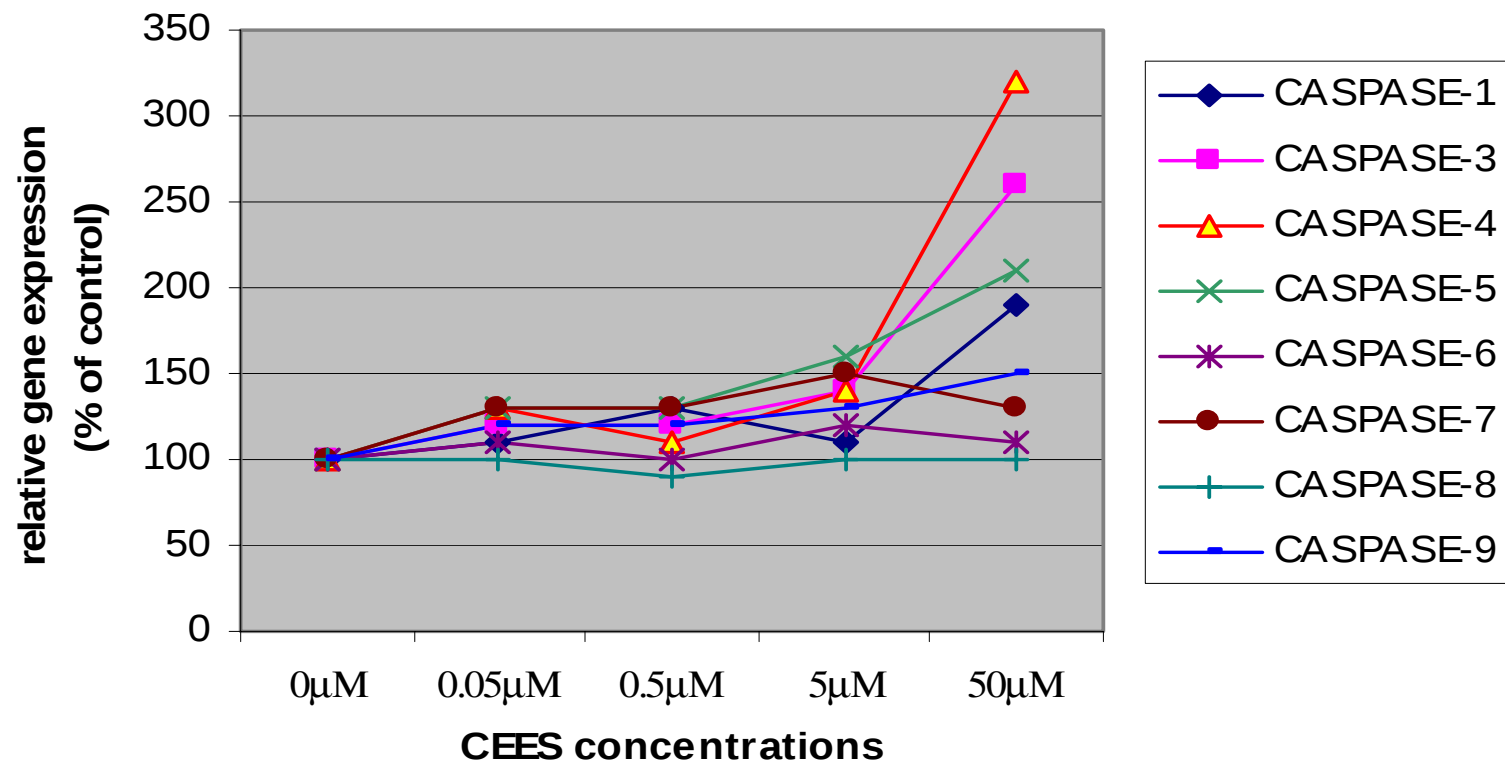


cDNA array

50 μ M CEES/24 hrs/Jurkat cells



Microarray reveals most caspases expression was induced by CEES

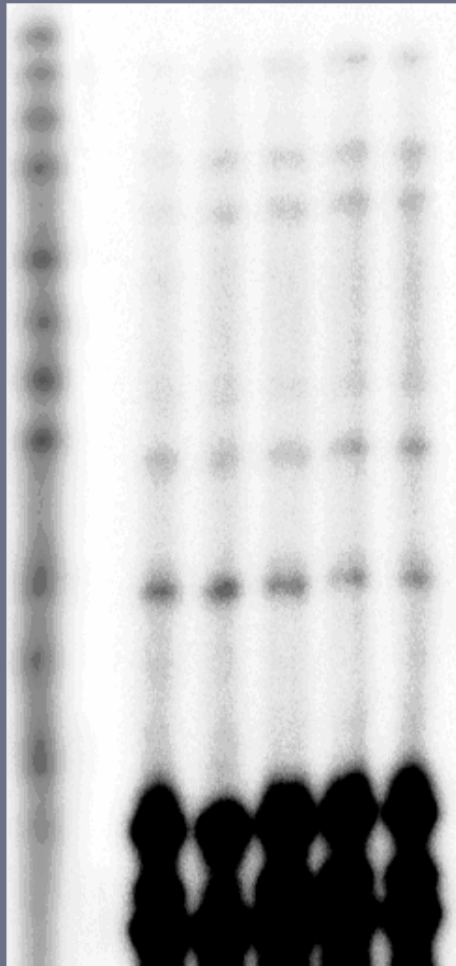


Array data was validated by RPA, RT-PCR, WB and activity assay

(A)

CEES[μ M] 0 0.05 0.5 5 50

435
390
350
312
256
238
210
192
156
141
124

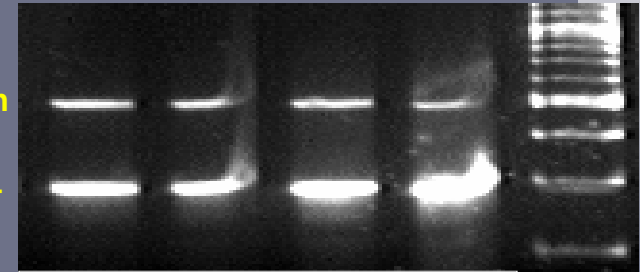


Unprotected
(nt)

(B)

β -actin

Caspase 4



(C)

CEES [μ M]

0 0.5 5 50

Caspase-3

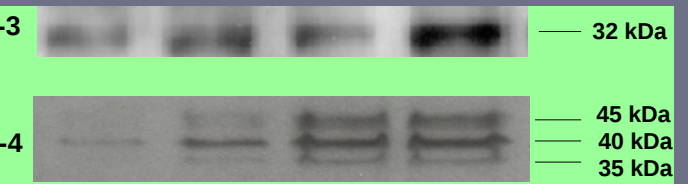
— 32 kDa

Caspase-4

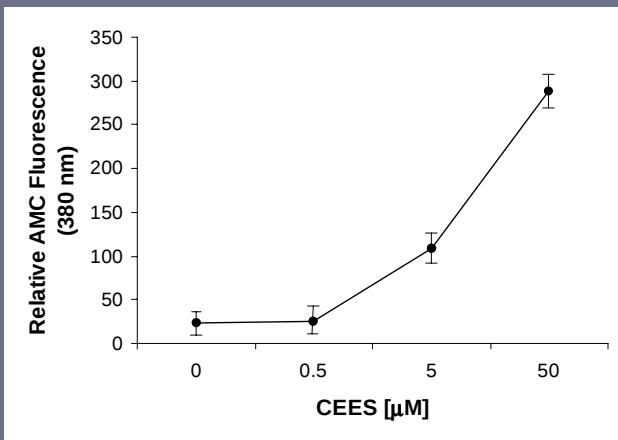
— 45 kDa

— 40 kDa

— 35 kDa

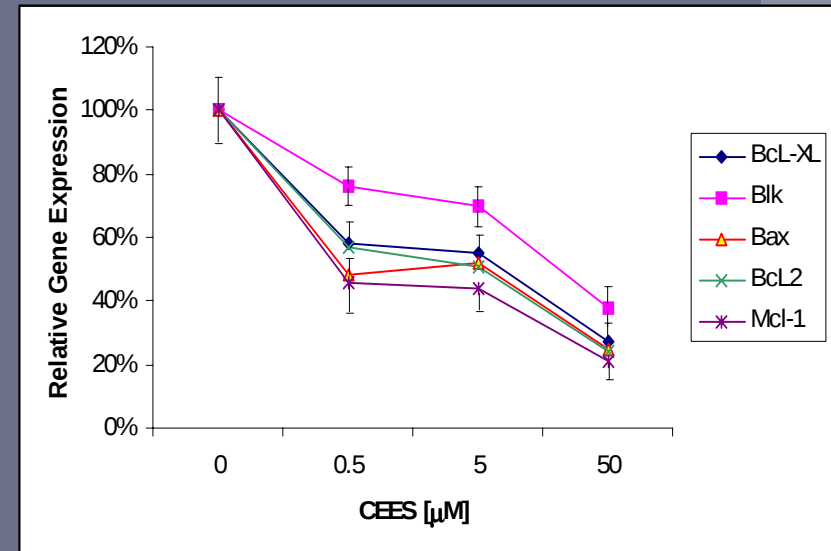
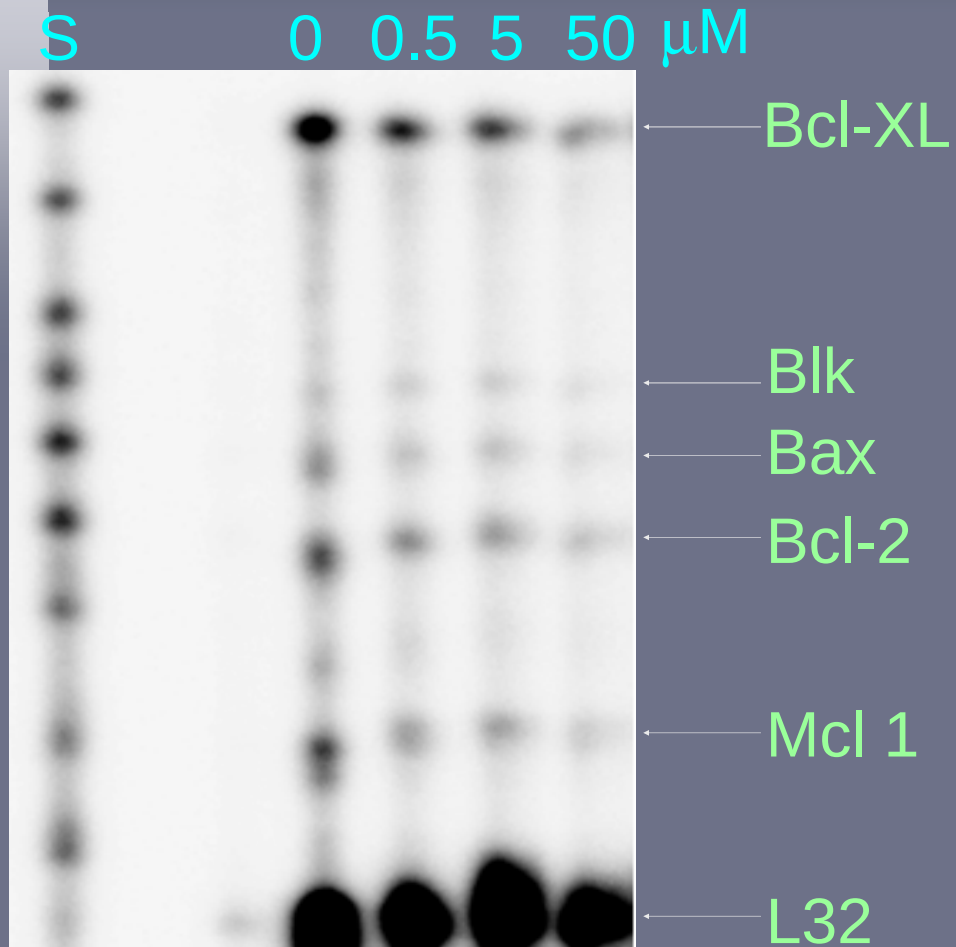


(D)



Caspase-3 enzyme Activity

CEES INHIBITED BCL FAMILY EXPRESSION

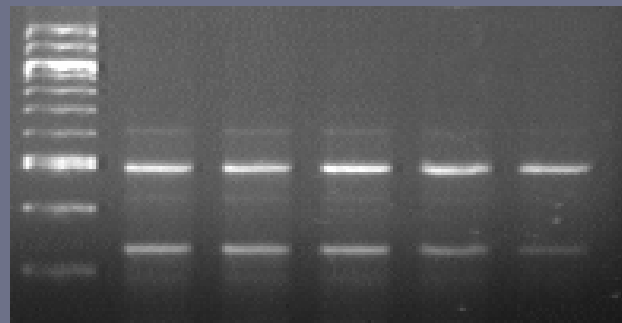


RNAse Protection

CEES Inhibited Akt/PKB expression

(A) RT-PCR

CEES[μ M] 0 0.05 0.5 5 50



β -actin

Akt

(B) Western Blotting

CEES [μ M]

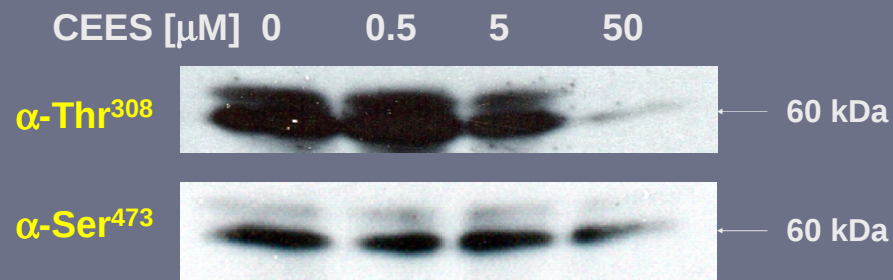
0 0.5 5 50



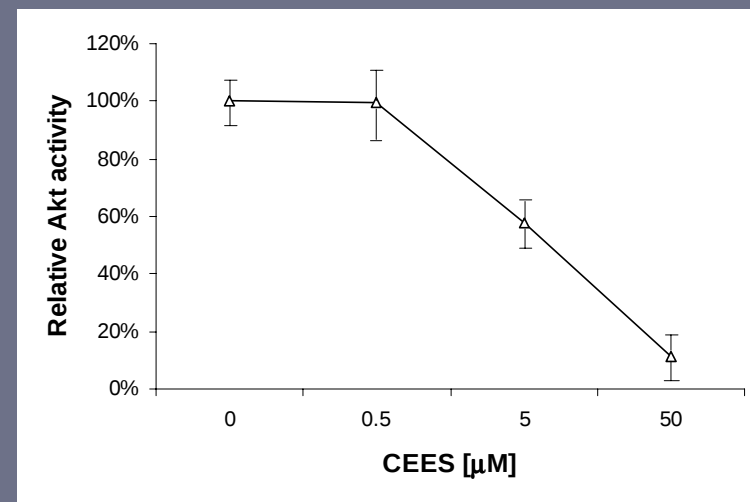
← 59 kDa

Phosphorylation and activities of Akt were affected by CEES

(A) Phosphorylation of Akt
(Western blotting)

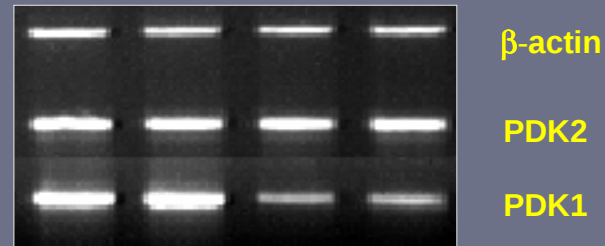


(B) Akt kinase activity assay

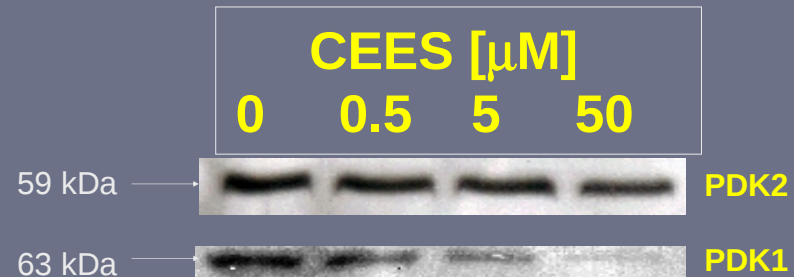


PDKs expression was affected by CEES

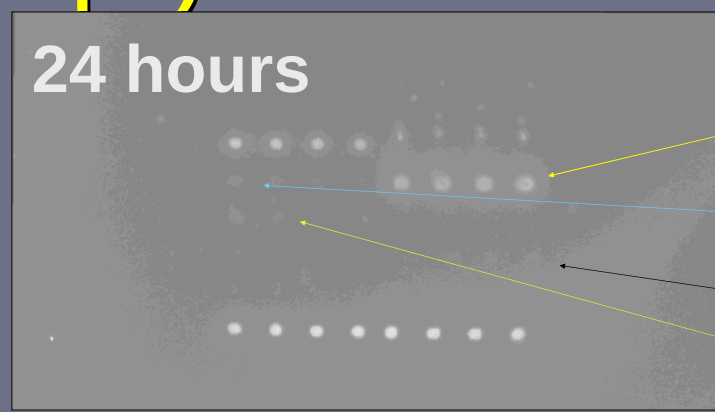
(A) RT-PCR



(B) Western Blotting



Cytokines Were Induced in Late Stage of CEES Treatment (200 μ M) in Jurkat cells



IL-8

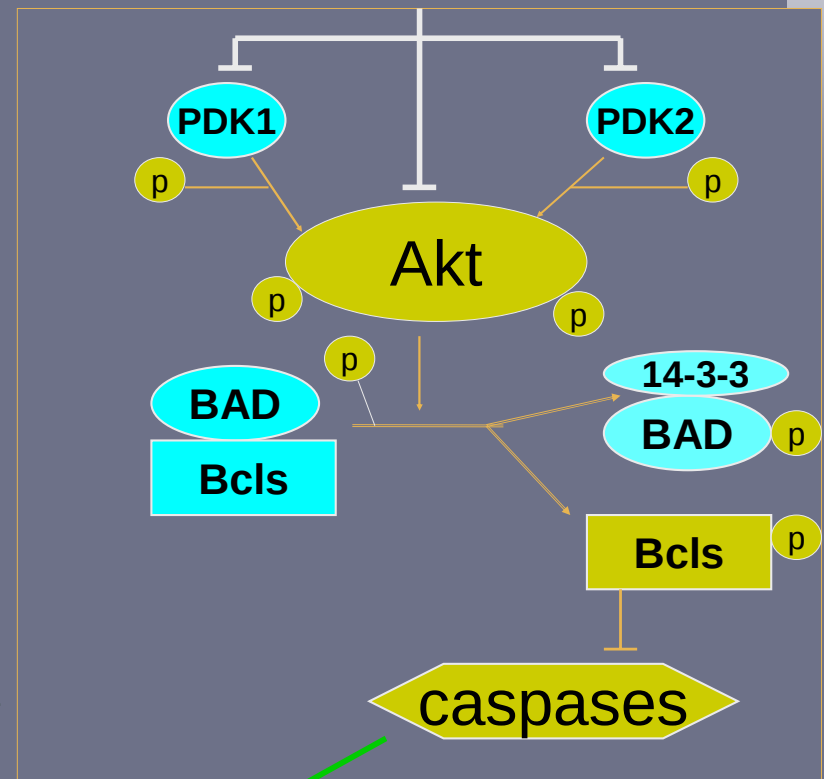
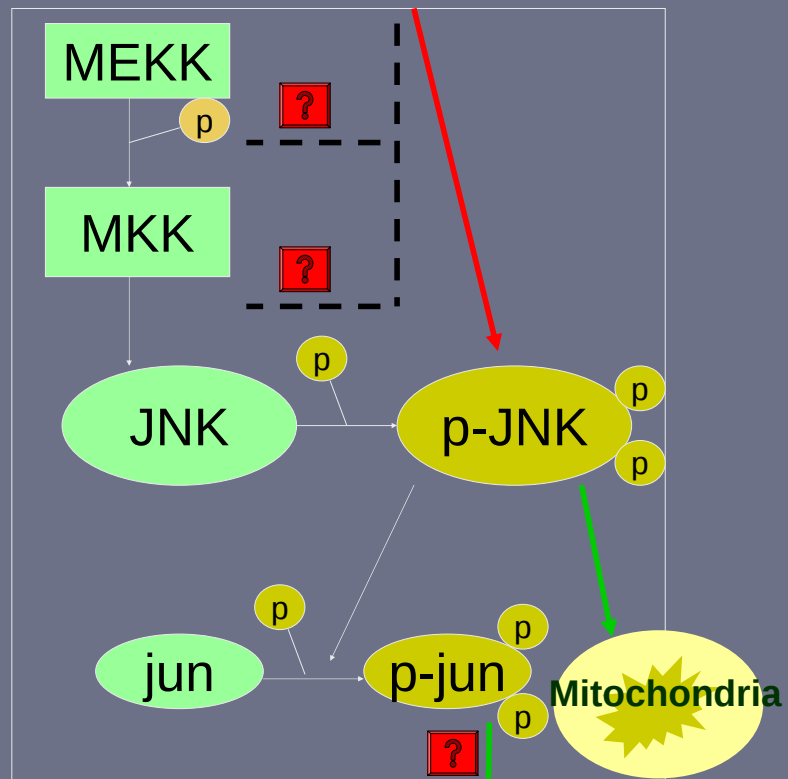
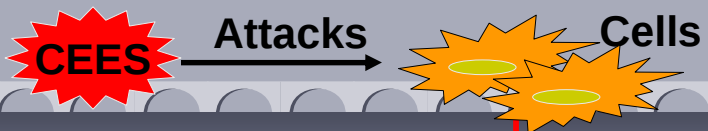
TNF- α

IL-1B

IL-10

Staining	Staining	Staining	Staining	Staining	Staining	Staining	Staining
TNF- α	TNF- α	TNF- α	TNF- α	IL-8	IL-8	IL-8	IL-8
IL-10	IL-10	IL-10	IL-10	PBS	PBS	PBS	PBS
PBS	PBS	PBS	PBS	IL-1B	IL-1B	IL-1B	IL-1B
IL-4 (Rat)	IL-4 (Rat)	IL-4 (Rat)	IL-4 (Rat)	IL-6	IL-6	IL-6	IL-6
Staining	Staining	Staining	Staining	Staining	Staining	Staining	Staining

(Protein array)



Early Stage

Late Stage





Conclusion

- Sulphur mustard causes cell death via apoptosis:
- In early stage, It induces JNK activity and then triggers apoptosis pathway.
- In late stage, sulphur mustard attacks the Akt pathway, by inhibiting Akt transcription, translation, and post-translation modification. Concomitantly, the anti-apoptotic genes, Bcl family, were down-regulated, in sharp contrast to the striking up-regulation of some death executioner genes, caspases 3, 8, 6 and 5.
- Sulphur mustard also induces some cytokines expression.
- Take together, sulphur mustard induces apoptosis by inhibiting the cellular survival factors which suppresses the expression of caspases.