This student paper was written as an assignment in the graduate course

Free Radicals in Biology and Medicine

(77:222, Spring 2005)

offered by the

Free Radical and Radiation Biology Program
B-180 Med Labs
The University of Iowa
Iowa City, IA 52242-1181
Spring 2005 Term

Instructors:
GARRY R. BUETTNER, Ph.D.
LARRY W. OBERLEY, Ph.D.

with guest lectures from:
Drs. Freya Q . Schafer, Douglas R. Spitz, and Frederick E. Domann

The Fine Print:

Because this is a paper written by a beginning student as an assignment, there are no guarantees that everything is absolutely correct and accurate.

In view of the possibility of human error or changes in our knowledge due to continued research, neither the author nor The University of Iowa nor any other party who has been involved in the preparation or publication of this work warrants that the information contained herein is in every respect accurate or complete, and they are not responsible for any errors or omissions or for the results obtained from the use of such information. Readers are encouraged to confirm the information contained herein with other sources.

Oxidative Control of DNA Repair:
Radiation effects on Ku

Michael J. Hitchler
Department of Radiation Oncology
Free Radical and Radiation Biology Program
The University of Iowa
Iowa City, IA 52242-1181

Irradiation of cells

- Radiation changes the redox state of cells
- DNA damage by radiation is free radical driven
  - Base damages
  - Strand breaks
  - DNA/protein cross-links
- Mechanisms exist to repair the damage
  - DNA repair enzymes and proteins
  - Glutathione powers the reactions through redox

DNA repair

- Radiation activates ATM/ATR kinases
  - Activates cell cycle checkpoints
    - Stabilization of p53
  - DNA repair proteins
  - Replication Protein A, Rad51, DNA associated protein Kinase (DNAPK)

Khanna, 2001

- Divided into four major subclasses
  - Homologous recombination (HR)
  - Nucleotide excision repair (NER)
  - Base excision Repair (BER)
  - **Non-homologous end joining (NHEJ)**

DNA repair

- Complex and concert process
  - Many classes of proteins involved
    - Identified by radiation sensitivity
    - Human diseases sensitive to UV-light

Khanna, 2001

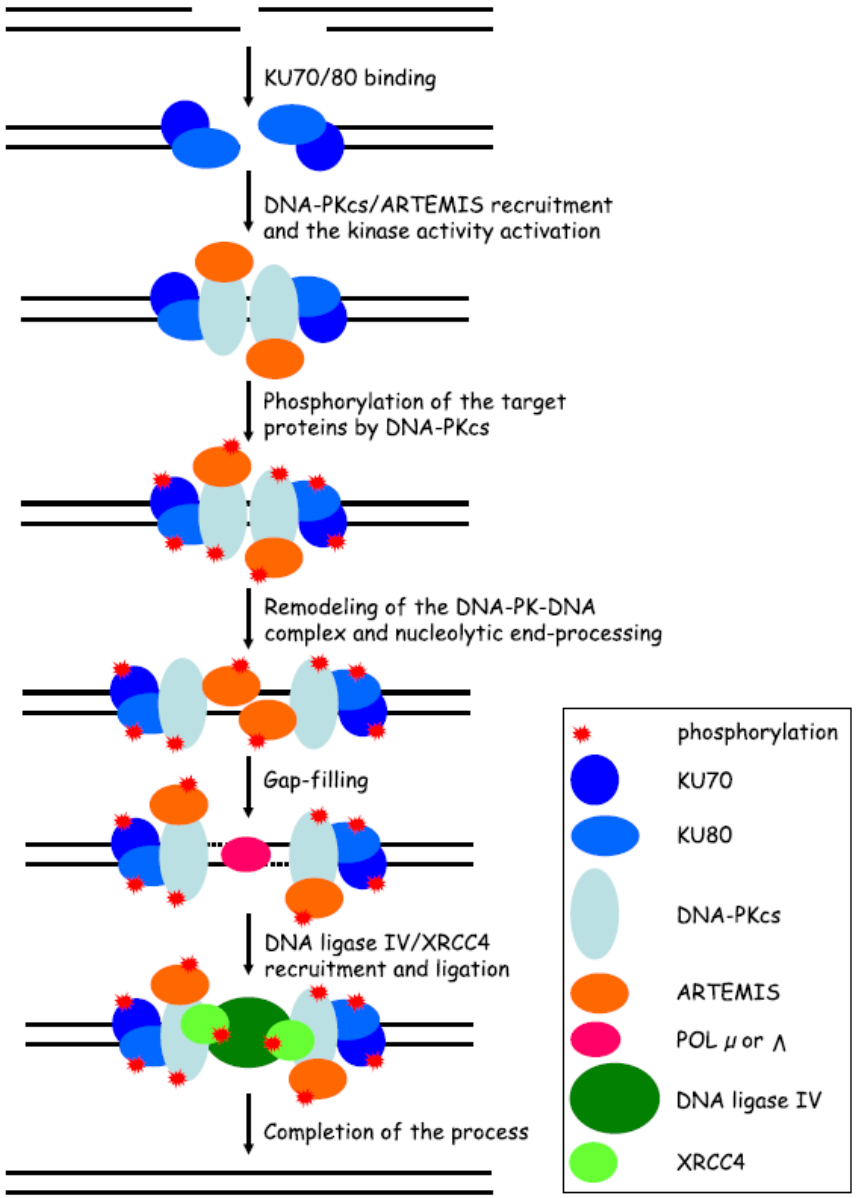
- Proteins are redox regulated
  - Zinc Finger Motifs
  - Oxidizable cysteines

Ku70/80 heterodimer

- Structure of Ku
  - Consists of 70 kDa and 80 kDa subunits
  - Interacts with DNA-PKcs, DNA ligase IV and DNA polymerases
  - Forms part of the telomerase complex
  - **Ku70/80 contains 12 oxidizable cysteines** (Zhang *et al.* 1993)
- Functions of Ku70/80
  - Non-homologous end joining
    - ATP-dependent DNA helicase
    - Protects DNA ends from degradation
    - Recruits DNA-PKcs when bound to DNA ends
    - Recruits DNA ligase IV/XRCC4 to site of DSB
    - Stimulates DNA ligase IV/XRCC4-mediated ligation
  - Telomere maintenance
    - Interacts and recruits hTERT

Dudasova, 2004

Ku in NHEJ



Ku Functions in a concerted process

1) Binds dsDNA breaks
2) Recruits proteins with enzymatic function
3) Repair of DNA lesion

Dudasova, 2004

Dogma and heresy

- Dogma: Radiation DNA damage causes cell death
  - Thought to be the primary target of radiation in cells
  - Generates many irreparable damages
    - ssDNA breaks
    - dsDNA breaks
    - DNA protein cross-links
- Heresy: Radiation inactivates proteins by redox
  - Enzymes in metabolic pathways are inactivated
    - Causes depletion of cellular GSH pool
  - DNA repair proteins no longer function because they are oxidized
- Oxidized repair means you can no longer repair DNA
  - Perhaps this is the major cause of death

Methods

- Use Chinese hamster ovary cell lines
  - Wild type (K1)
    - Normal cell control in experiments
  - G6PD null (E89)
    - Mutant in first step of oxidative pentose phosphate pathway.
    - Have less NADPH and lower GSH after stress
  - Transfected G6PD null (A1A or)
    - Used to as a control to demonstrate mutation is specific for G6PDH

Ayene *et al.,* 2002

- Apply hydroxyethyldisulfide (HEDS)
  - Stress cells 1 h with 5 mM HEDS
  - Consume GSH and cause oxidative stress

Ayene *et al.,* 2002

Methods

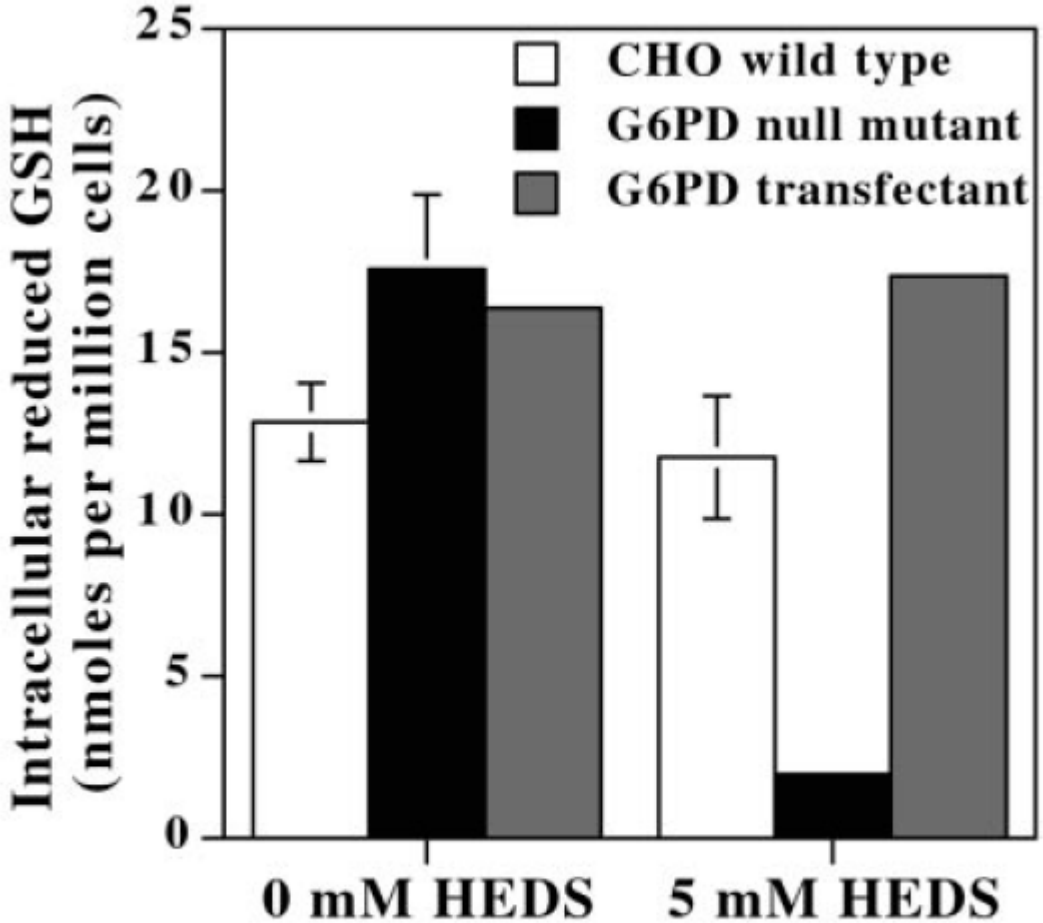
- Measure Ku DNA binding
  - Gel shift assay specific for Ku70/80
    - Labeled dsDNA probe
    - Specificity by adding cold ssDNA
  - Use nuclear extracts from cells
    - Correlate biological effects with Ku functional status

Ayene *et al.,* 2002

- Clonogenic survival after radiation

Biaglow *et al.,* 2003

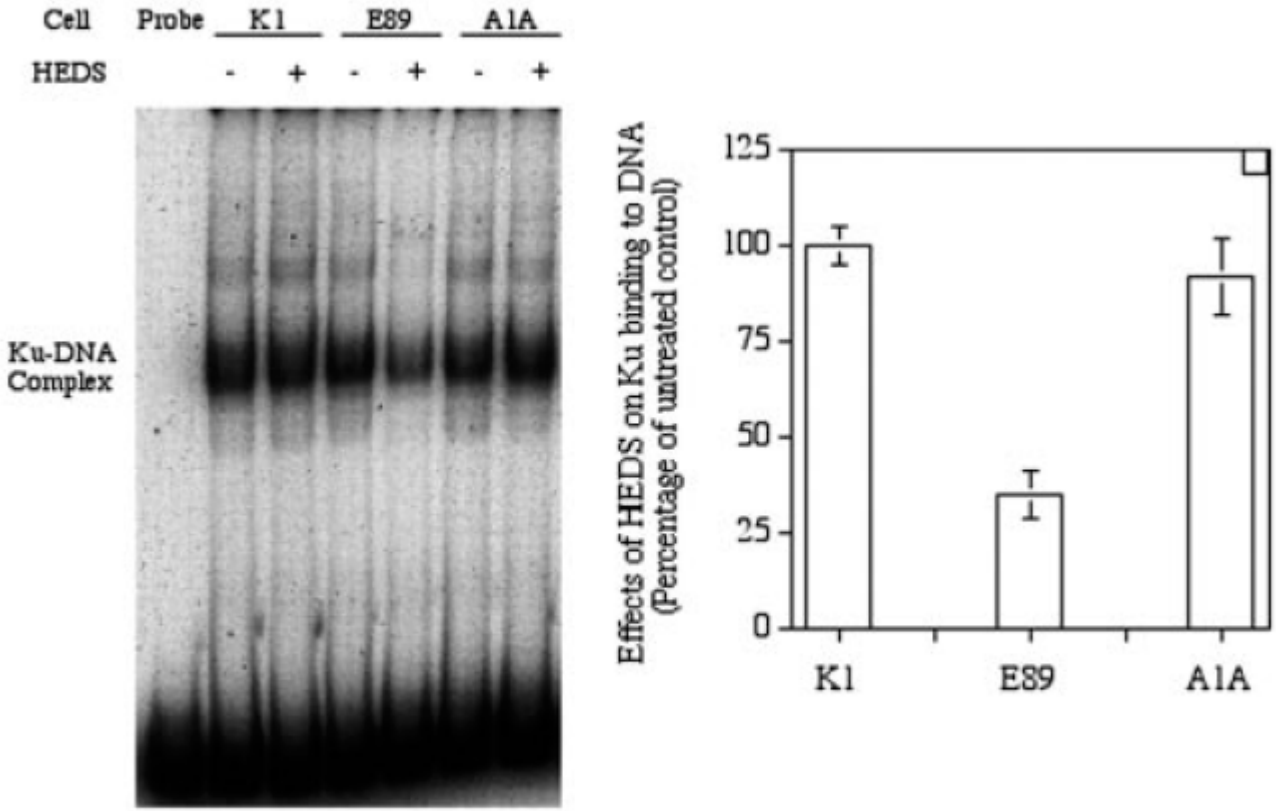
GSH depletion in G6PD mutants



All three CHO cell lines have comparable GSH levels. G6PDH mutant cells cannot replenish GSH after oxidative stress with HEDS. This is expected since the levels of NADPH in these cells would be less since the oxidative pentose phosphate pathway is inactivated. Also the mutant that has been transfected with a functional G6PDH gene is functioning properly.

Ayene *et al.,* 2002

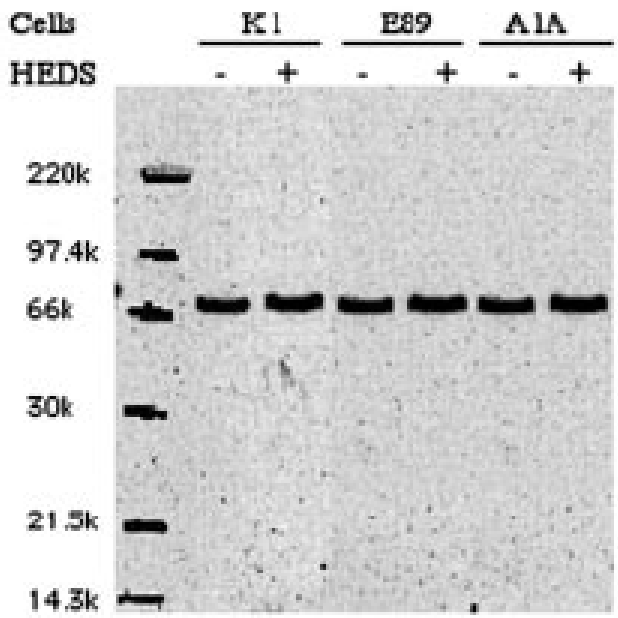
Redox influences Ku DNA binding

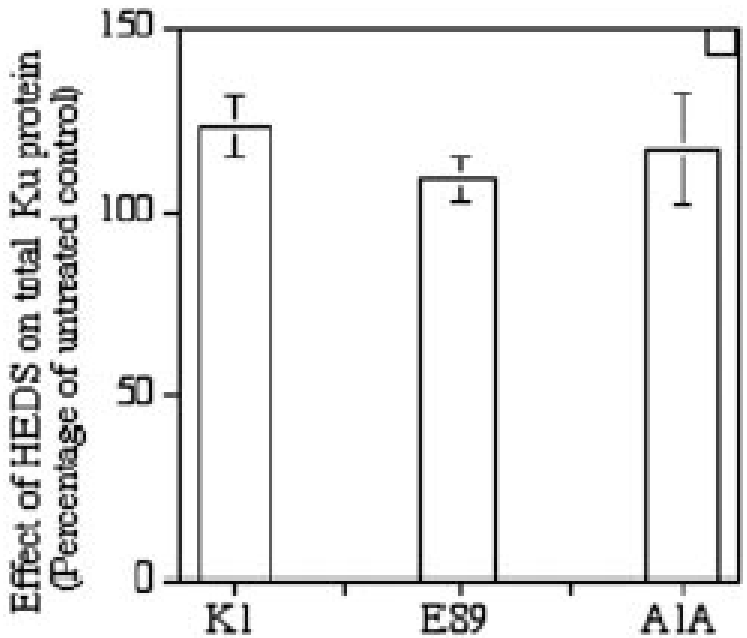


Using nuclear extracts from the different cell lines we see that they all have similar levels of Ku DNA binding by gel-shift. Oxidative stress by HEDS reduces the binding of Ku70/80 to 25% of the untreated control. Wild type and transfected control cells have no significant change in their binding activity. This is crucial because it correlates cellular oxidative state with Ku DNA binding.

Ayene *et al.,* 2002

GSH depletion in G6PD mutants

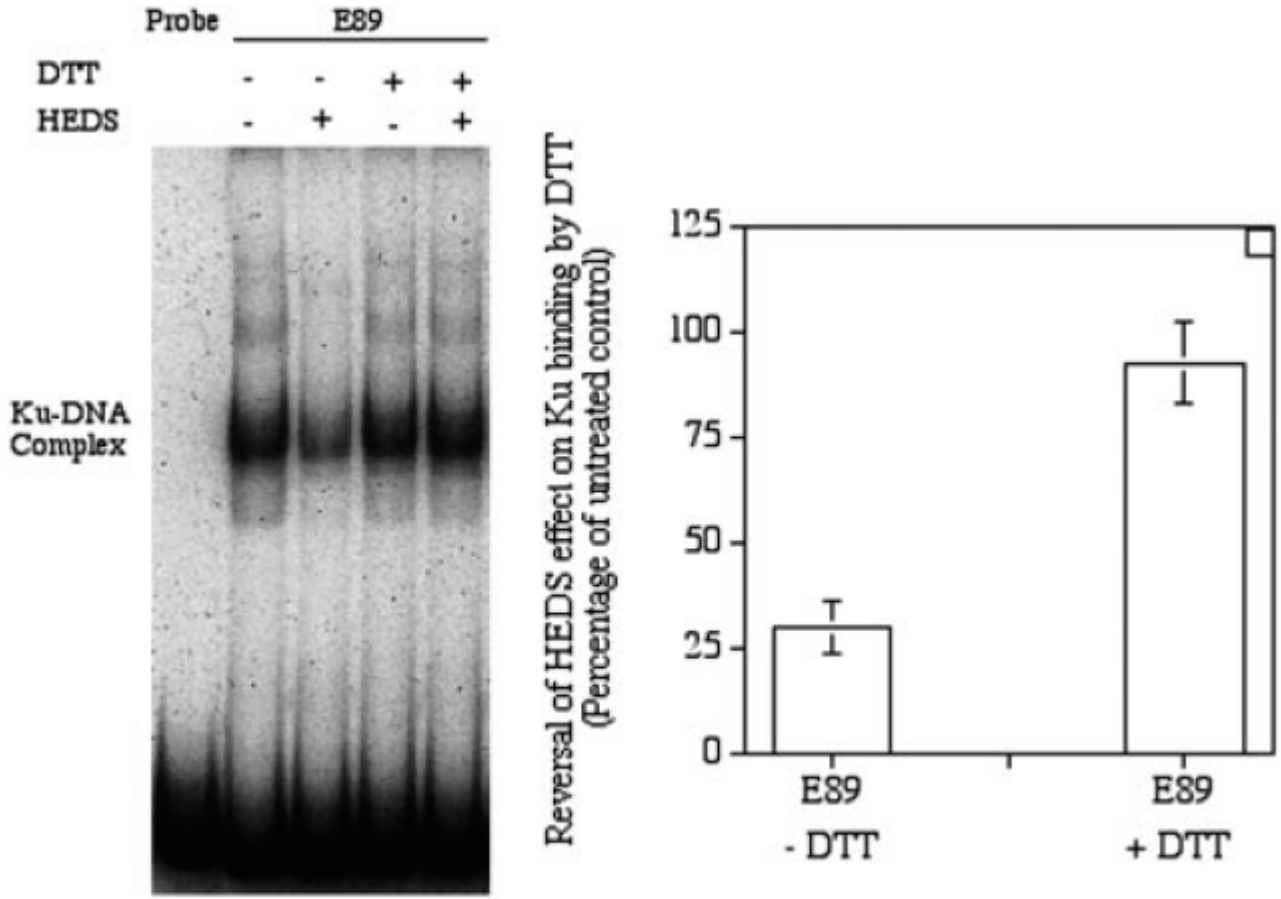




HEDS treatment does not significantly influence the level of Ku70 protein in CHO cells.

Ayene *et al.,* 2002

DTT restores Ku DNA binding



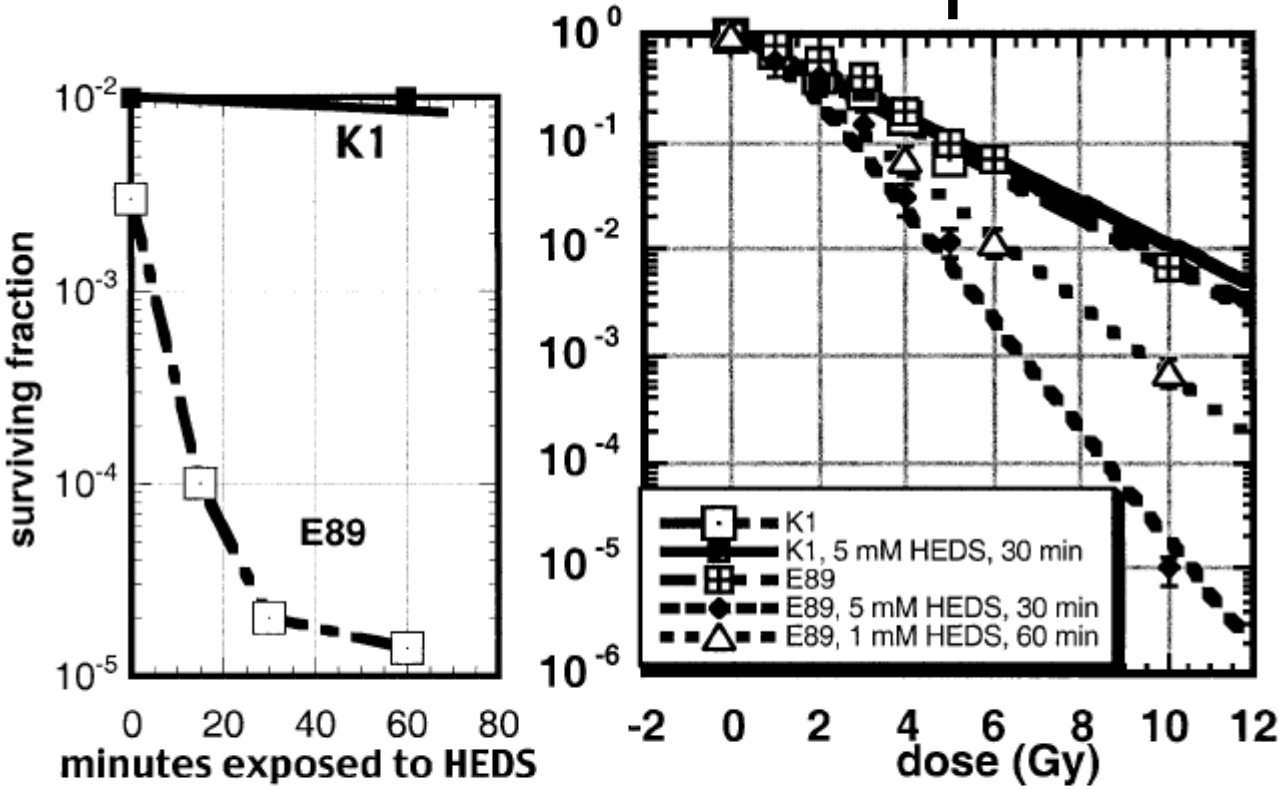
Is the oxidative inactivation of Ku by HEDS reversible? Addition of the reducing agent DTT increased Ku DNA binding. This means that redox is controlling Ku70/80 DNA binding in cells and therefore has influence on DNA repair in cells.

Ayene *et al.,* 2002

Summary

- Treatment of HEDS depletes cellular GSH
  - Induces oxidative stress
  - Decreases the ability of Ku to bind dsDNA
- HEDS inactivation is reversible
  - DTT restored Ku's dsDNA binding
- Strong evidence that Ku70/80 is redox regulated

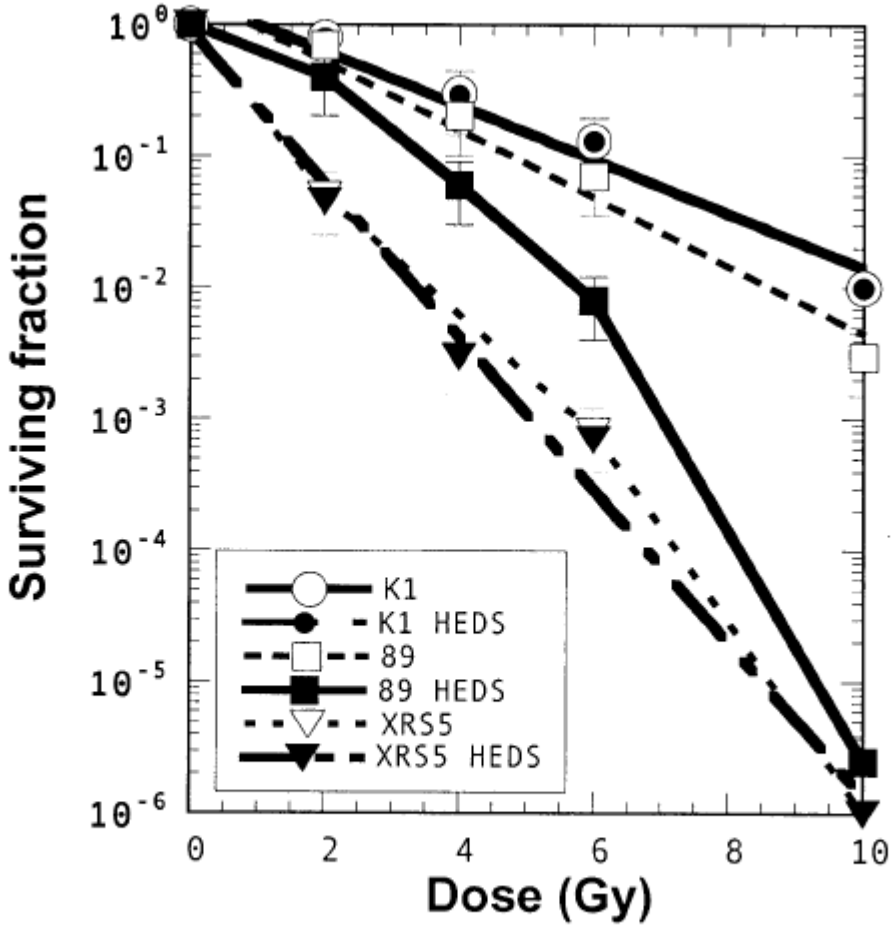
Cell survival: oxidized proteins



The oxidative state of the E89 cells makes them more sensitive to radiation. Therefore the Ku70/80 protein is less functional ad cell survival is decreased because double stranded breaks cannot be repaired. We can start to see how redox and DNA repair are coupled.

Biaglow *et al.*, 2003

Cell survival: oxidized proteins



The radiation sensitivity of E89 cells was determined by comparing it to wild type and the radiation sensitive XRS5 cell line. We see that upon addition of HEDS the E89 cells a have a sensitivity between wild type K1 and XRS5. Therefore redox state must be important in maintaining DNA repair mechanisms

Biaglow *et al.,* 2003

Summary

- HEDS + γ-radiation
  - Decreases cell survival of E89 mutants
  - Links redox state and DNA repair

- DsDNA breaks might be causing death
  - Increased radiation sensitivity
  - Ku70/80 would be inactivated

Observation summary

- Redox regulation of DNA repair proteins
  - Changes oxidation state of proteins
    - Now have decreased DNA binding
  - Controlled via key cysteine residues
    - Zinc finger motifs, outer cysteines
- Radiosenstized cells
  - Oxidized cells die faster
    - Similar to DNA repair mutant
- Mechanism of cell death
  - Links DNA repair protein redox with cell death

Other implications

- Redox regulation of DNA repair
  - Metabolic link to genetic instability
- Free radical theory of aging
  - Free radicals make you age
    - Metabolism slows could this decrease DNA repair?
    - Accumulation of DNA mutations
      - Increased cancer
  - Old cells unable to maintain telomeres
    - Telomere length influences cell senescence
      - Aging clock is set with metabolism controlling telomere lenght

References

1. Khanna, K. K., and Jackson, S. P. (2001) *Nat Genet* **27**(3), 247-254.
2. Zhang, W. W., and Yaneva, M. (1993) *Biochem J* **293 (Pt 3)**, 769-774.
3. Dudasova, Z., Dudas, A., and Chovanec, M. (2004) *FEMS Microbiol Rev* **28**(5), 581-601.
4. Ayene, I. S., Stamato, T. D., Mauldin, S. K., Biaglow, J. E., Tuttle, S. W., Jenkins, S. F., and Koch, C. J. (2002) *J Biol Chem* **277**(12), 9929-9935.
5. Biaglow, J. E., Ayene, I. S., Koch, C. J., Donahue, J., Stamato, T. D., Mieyal, J. J., and Tuttle, S. W. (2003) *Radiat Res* **159**(4), 484-494.