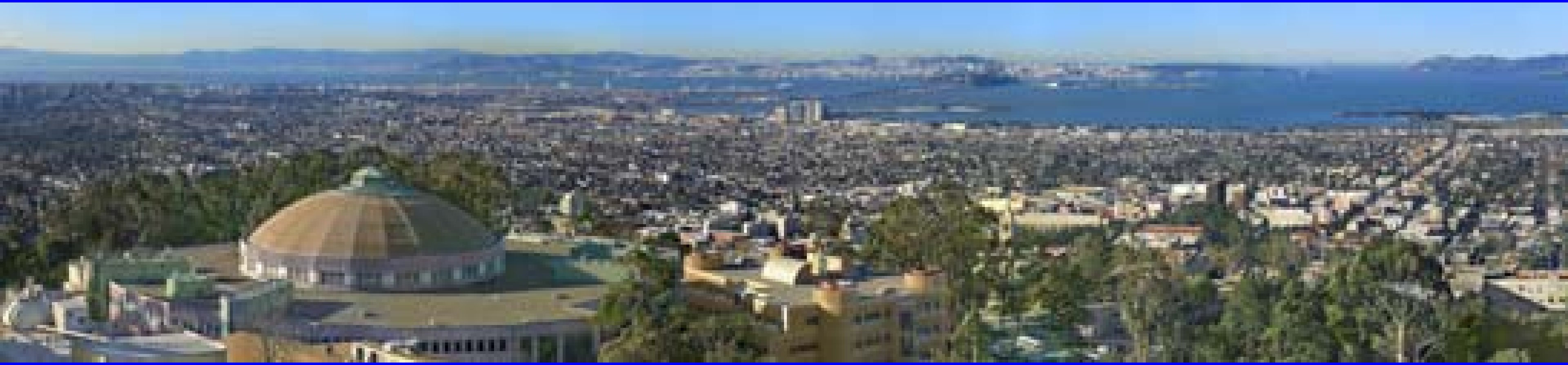
47th Annual Meeting Scientific and Policy
Challenges of Particle Radiation in
Medical Therapy and Space Missions
Bethesda, MD

What makes particle radiation so effective?

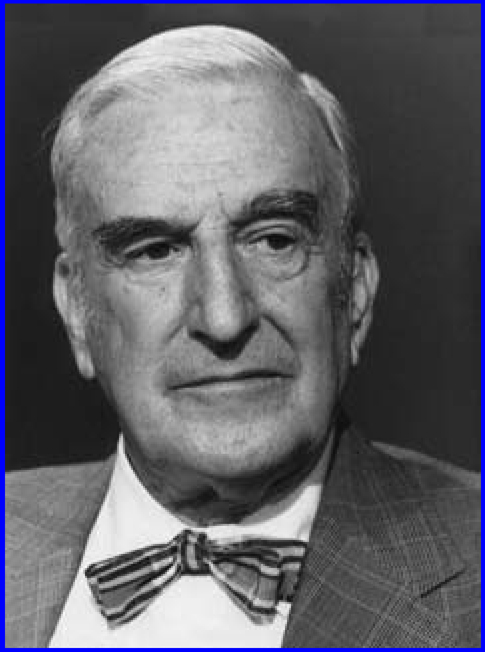


Eleanor A. Blakely

35th Lauriston S. Taylor Lecture

7 March 2011

Lauriston S. Taylor
1902-2005



- "Mr. Radiation Protection"
- Member of ICRU & ICRP
- Founder & 1st President of NCRP in 1929
- Congress granted NCRP a federal charter in 1946
- Career of extraordinary diversity
- Superb administrator and diplomat

Lauriston Taylor, D.Sc.

- Author of a manuscript copyrighted 1990* entitled:

  “ *What you need to know about radiation*”
  - *To protect yourself*
  - *To protect your family*
  - *To make reasonable social & political choices*

* http://www.physics.isu.edu/radinf/lst.htm

Lauriston Taylor, D.Sc.

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* http://www.physics.isu.edu/radinf/lst.htm

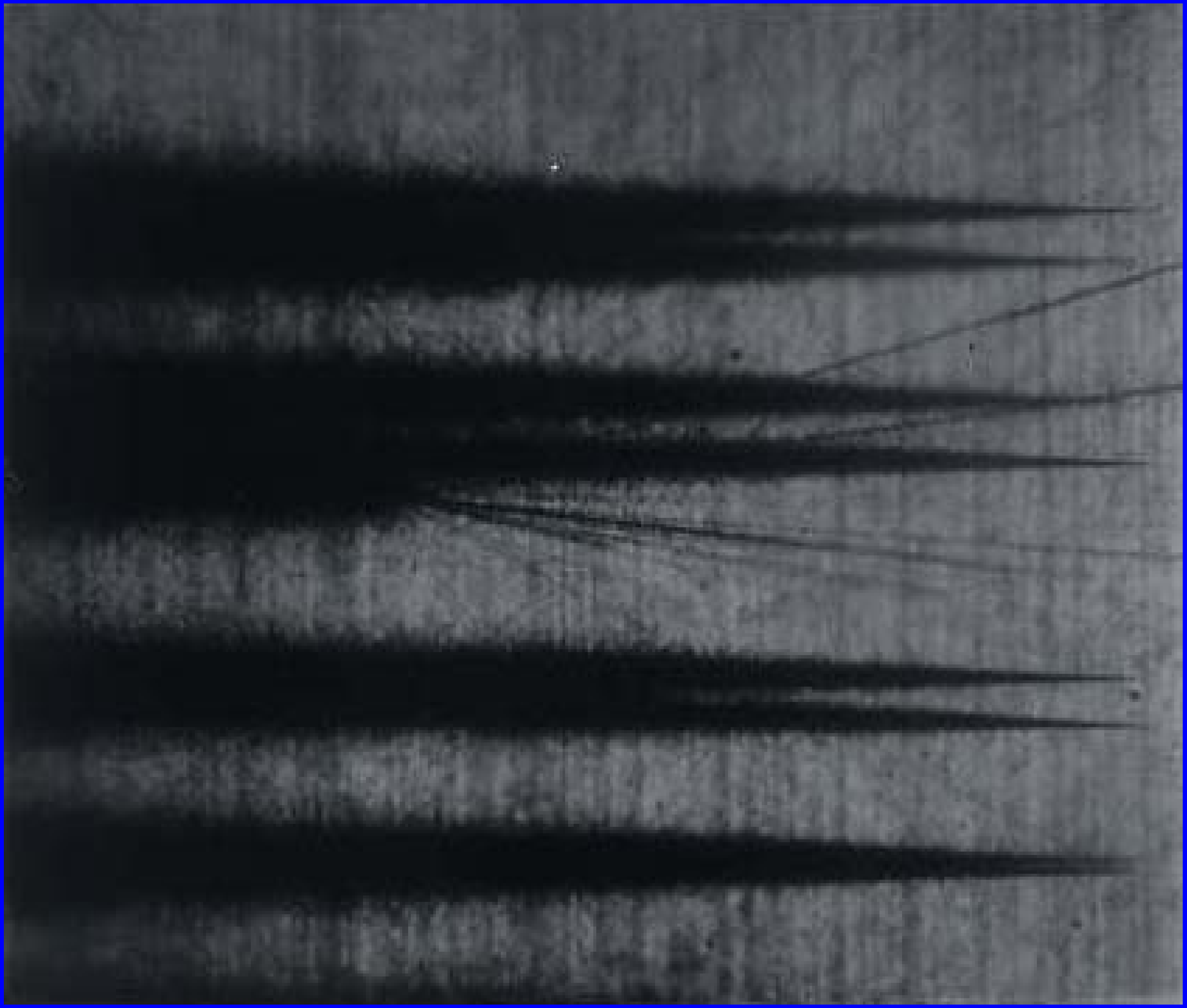
What makes particle radiation effective?

Overview

- Review underlying mechanisms of action of particle radiations that make them so effective from the perspective of 36 years in the lab & clinic
- How has our understanding grown?
- What do we still not understand?

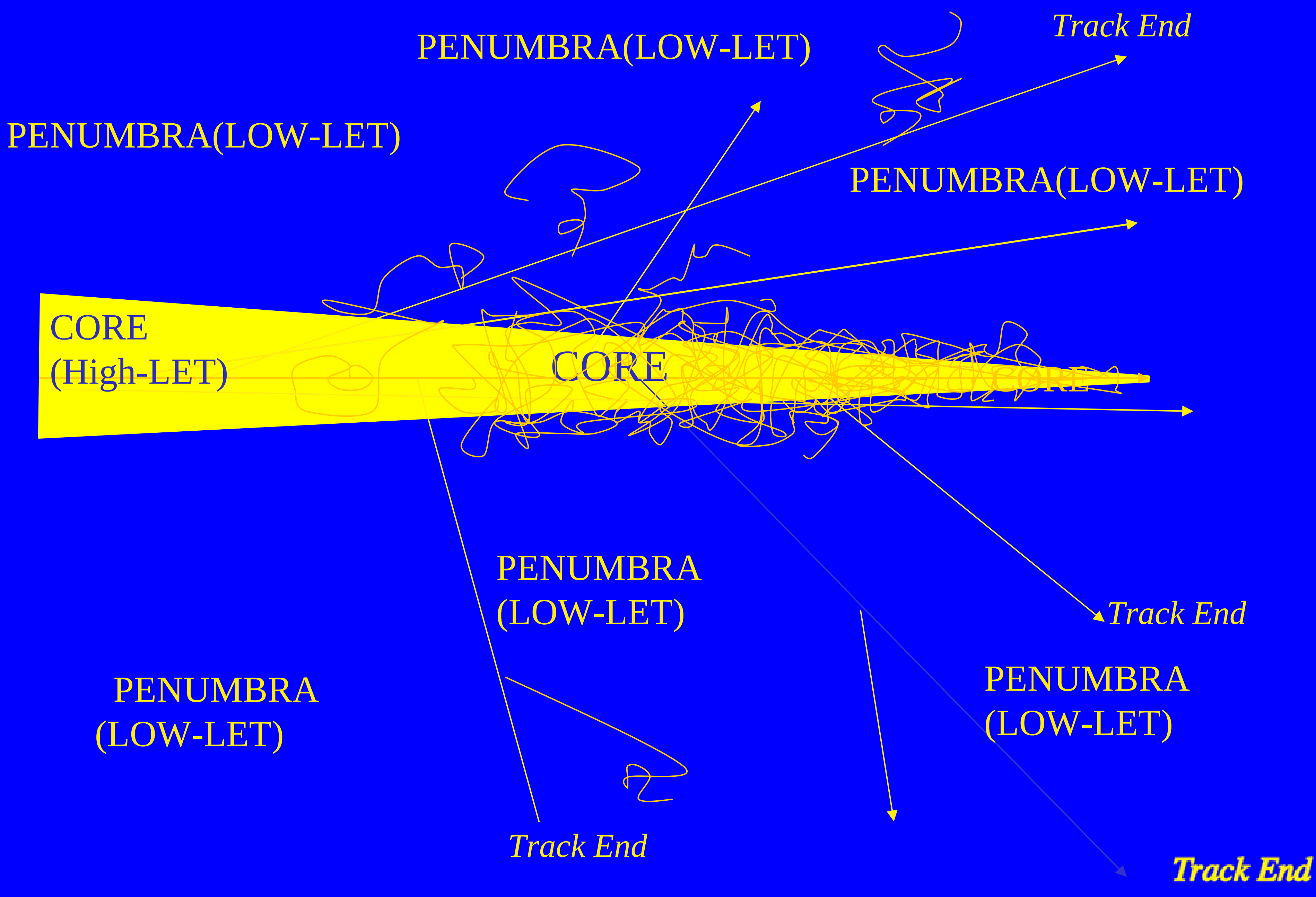
Dedication

HZE particle tracks in emulsion

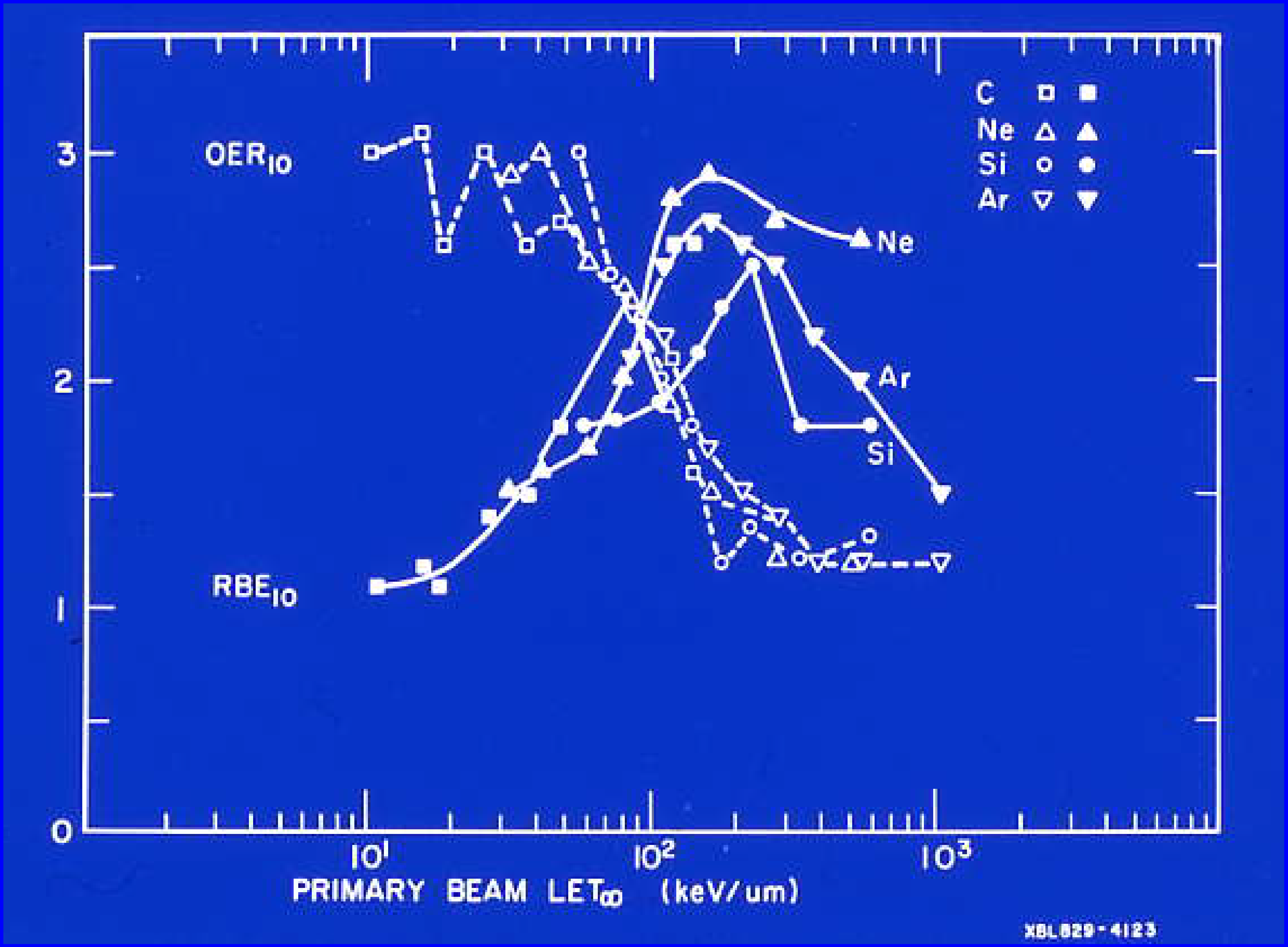


Heckman et al.

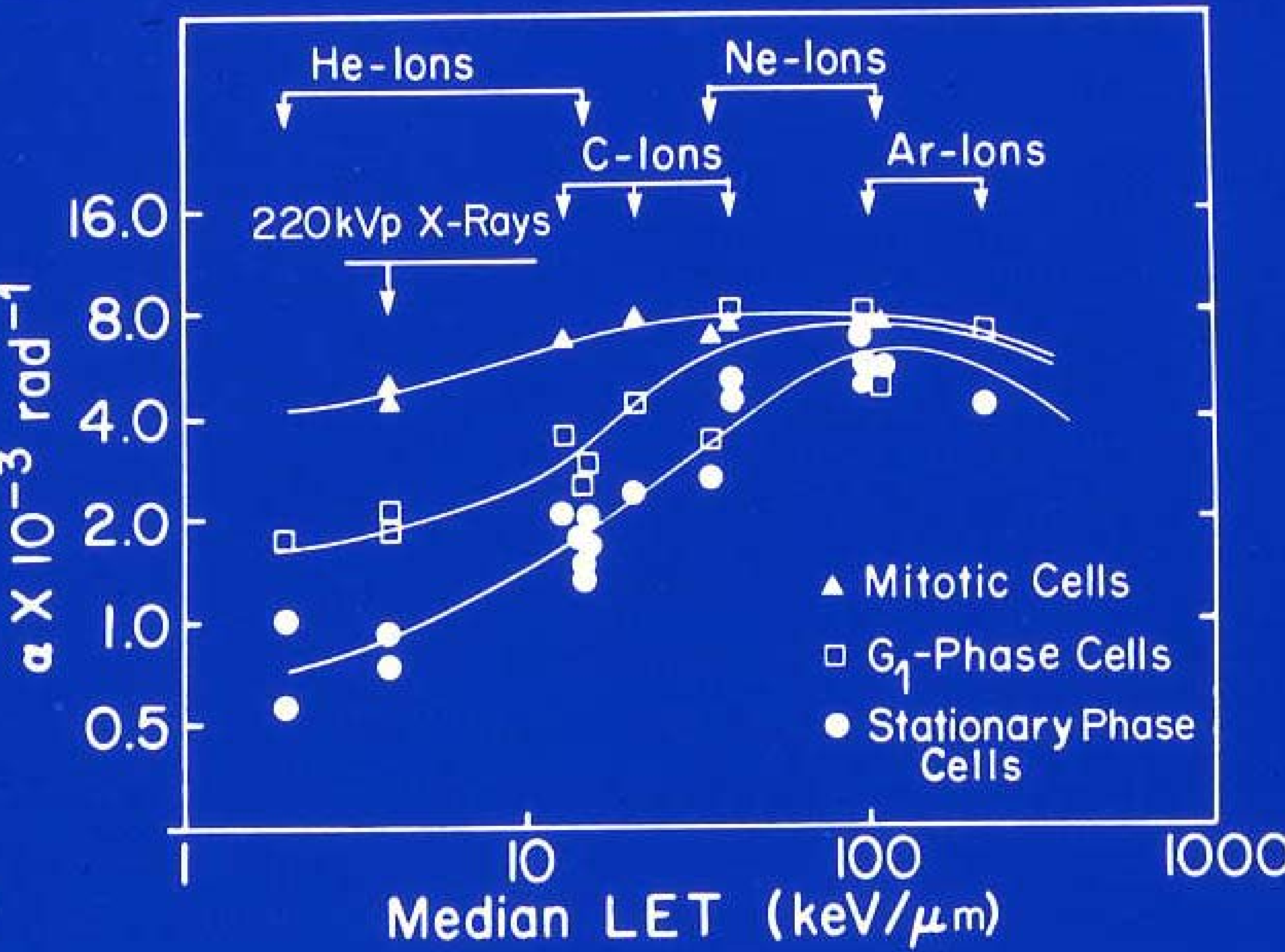
TRACK STRUCTURE OF HZE PARTICLES



LET-Dependence of HZE RBE & OER is Maximal Near 150 keV/mm

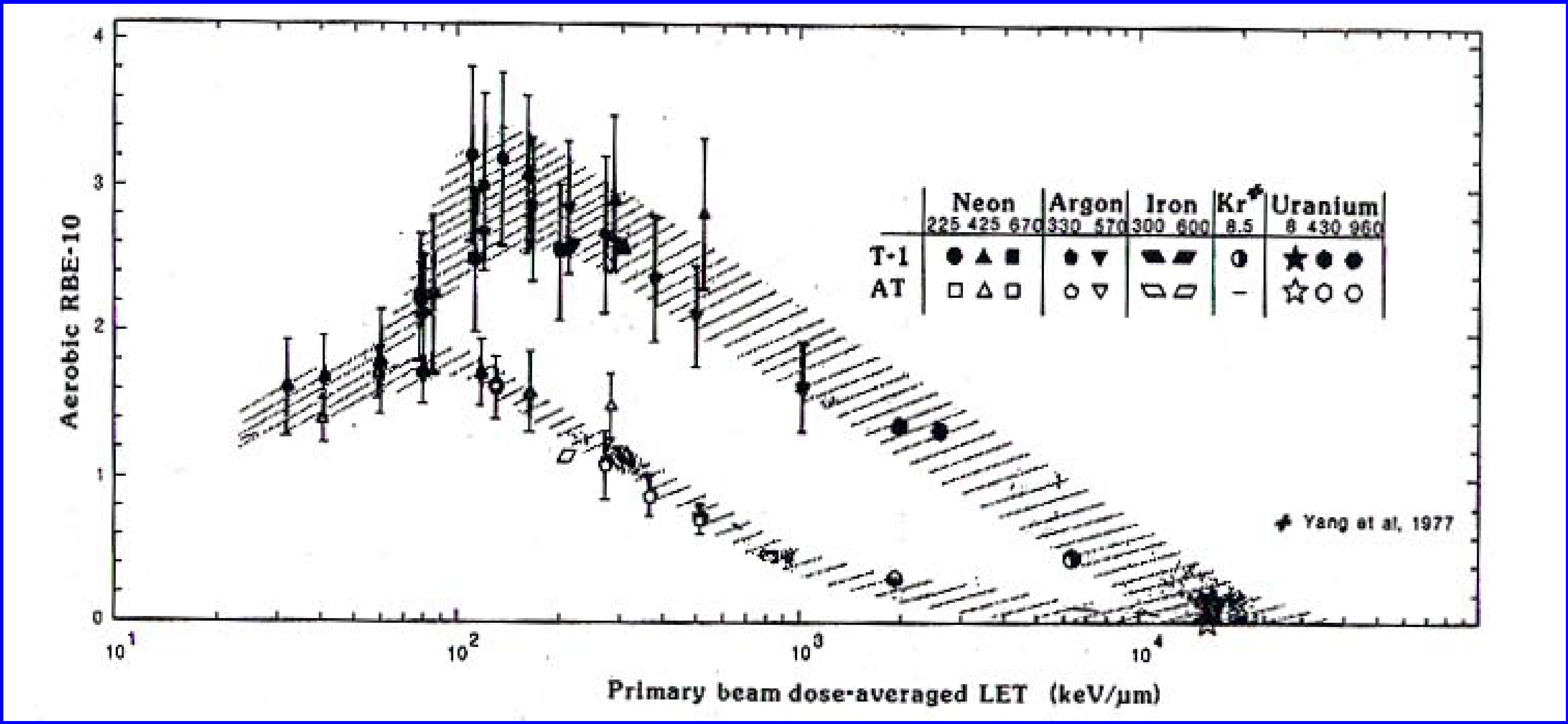


Blakely et al.



XBL 819-4965A

LET-Dependence of RBE for Normal & Radiosensitive Cells



Tobias & Blakely

Why the interest in particle therapy?

- Precision Therapy Conformed to Tumor
- Sparing of Normal Tissues
- Increased DNA Damage in Tumor
- Increased Effect on Hypoxic Tumors
- Less Repair of Sublethal and Potentially Lethal Damage in Cell Cycle
- Short Overall Treatment Course

Linstadt et al. 1991

Treatment Outcome Comparing Neon, Neutrons and Conventional Xray Therapy for Selected Types of Tumors

Tumor and Endpoint	Neon	Neutrons	Xray
Macroscopic Salivary Gland Ca			
(Long term local control)	61%	60-70%	25-36%
N=18			
Macroscopic Paranasal Sinus Ca			
(Long term survival)	69%	30+%	32-40%
(Long term local control)	69%	50-86%	N/A
N=10			
Macroscopic Soft Tissue Sarc			
(Long term local control)	56%	50-54%	30-50%
N=12			
Macroscopic Sarcoma of Bone			
(Long term local control)	59%	49-55%	21-33%
N=18			
Locally Advanced Prostate Ca			
(5 yr actuarial local control)	75%	77%	30-50%
N=12			

Reprinted from: Linstadt, Castro and Philips: Neon Ion Radiotherapy: Results of the Phase I-II Clinical Trial. Submitted to Int. J. Rad. Onc. Bio. Phys.

XBL 905-1897

Patients registered in Carbon Therapy at NIRS June 1994-March 2010

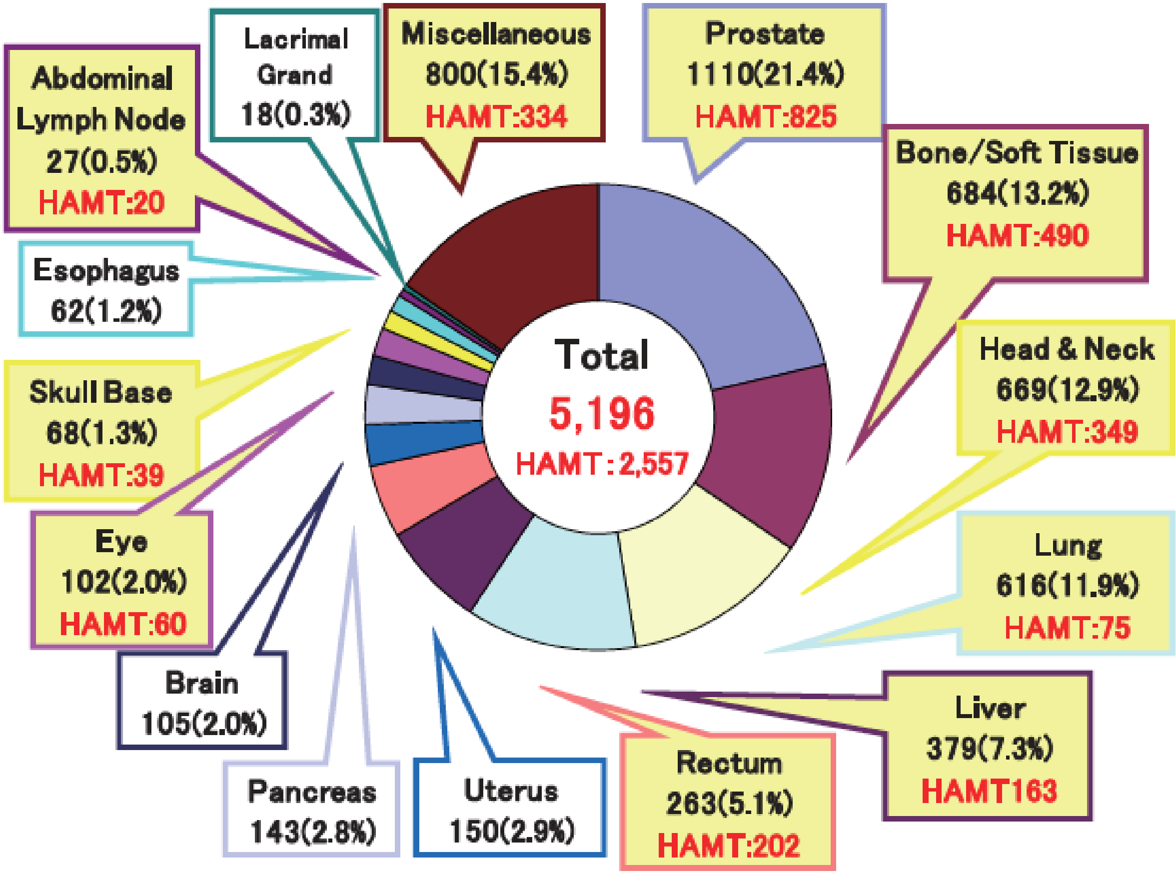
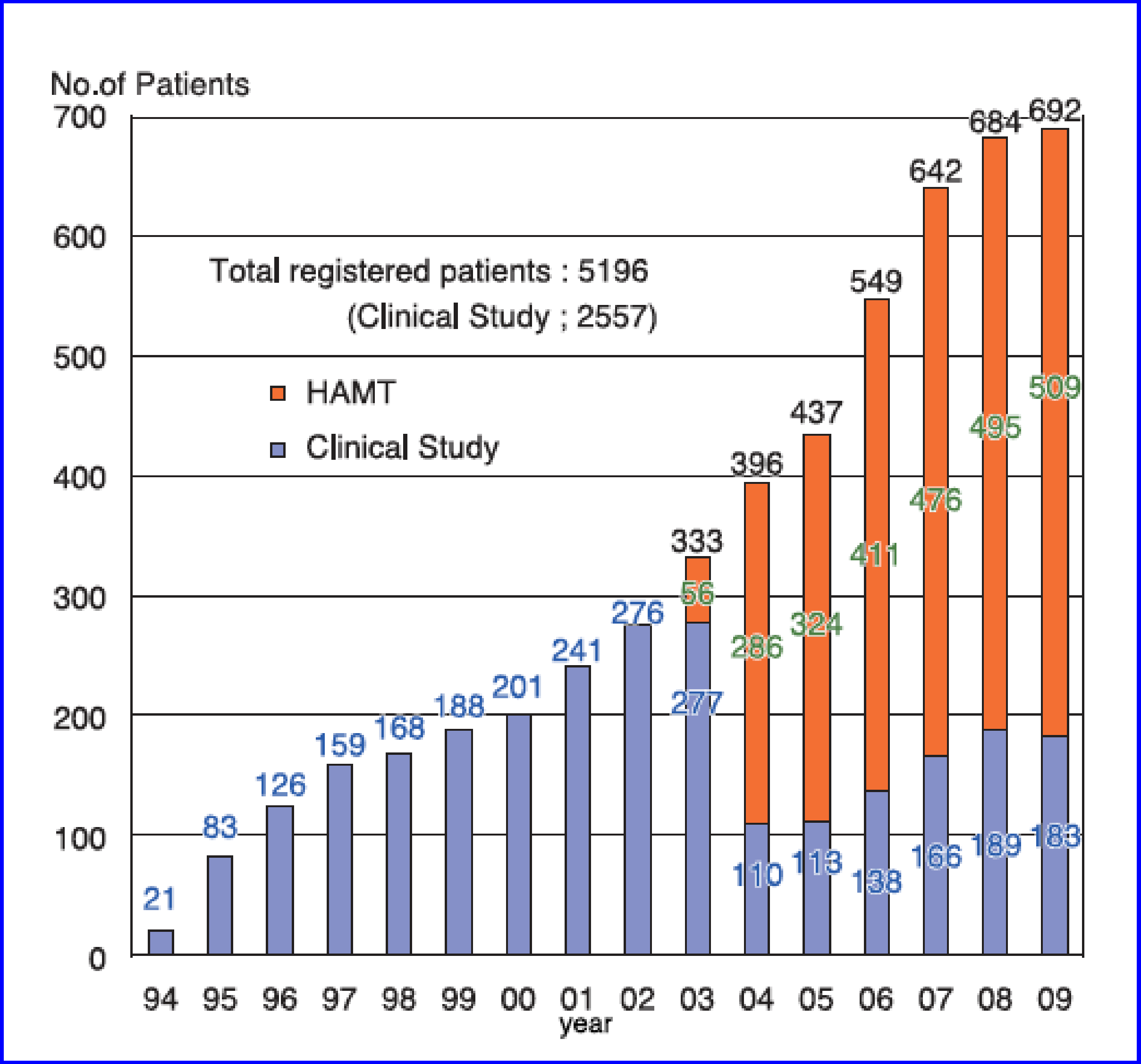


Fig. 3. Number of Patients registered in Carbon Ion Therapy at NIRS (Period: June 1994–March 2010). HAMT: Highly Advanced Medical Technology.

Okada et al., 2010

Patients registered in Carbon Therapy at NIRS June 1994-March 2010



Okada et al., 2010

Pt. Characteristics: 1st 80 patients at HIT

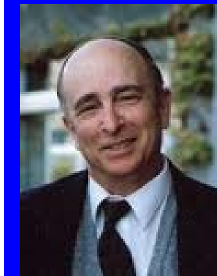
Table I. Patients' characteristics of the first 80 patients treated at the Heidelberg Ion Therapy center (HIT).

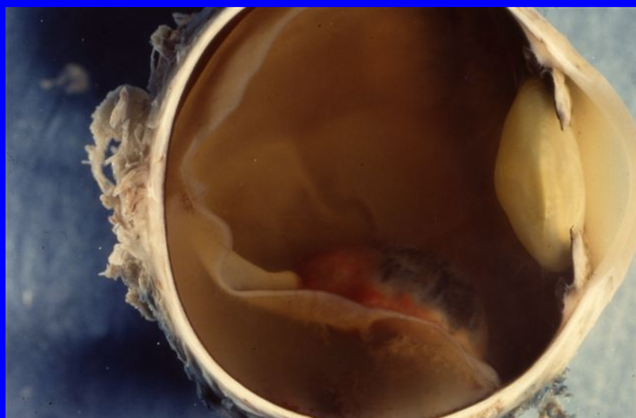
Indication	Number of patients n (%)
Skull Base	
Chordoma	9 (11%)
Chondrosarcoma	18 (22%)
Malignant Salivary Gland Tumors	29 (36%)
Astrocytoma	10 (13%)
pilocytic astrocytoma	1
WHO Grade II astrocytoma	2
anaplastic astrocytoma	1
primary glioblastoma	3
recurrent glioblastoma	3
Osteosarcoma	3 (4%)
skull and skull base	2
sacrum	1
Sacral Chordoma	5 (6%)
Other	6 (8%)
recurrent rectal cancer	2
nasopharyngeal cancer	1
rhabdomyosarcoma of the skull base	1
malignant melanoma of the paranasal sinus	1
chondrosarcoma of the left heel	1

Combs et al., 2010

PRECISION, HIGH DOSE RADIOTHERAPY: HELIUM ION TREATMENT OF UVEAL MELANOMA

WILLIAM M. SAUNDERS, PH.D., M.D.,[1,3] DEVRON H. CHAR, M.D.,[2] JEANNE M. QUIVEY, M.D.,[1] JOSEPH R. CASTRO, M.D.,[1,3] GEORGE T. Y. CHEN, PH.D.,[3] J. MICHAEL COLLIER, PH.D.,[3] AUDE CARTIGNY,[3] ELEANOR A. BLAKELY, PH.D.,[3] JOHN T. LYMAN, PH.D.,[3] SANDRA R. ZINK, PH.D.,[3] AND CORNELIUS A. TOBIAS, PH.D.[3]





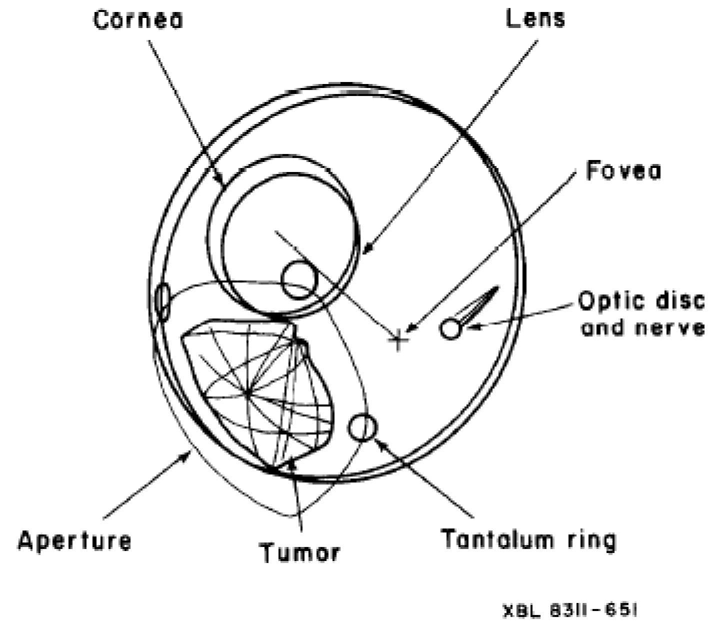
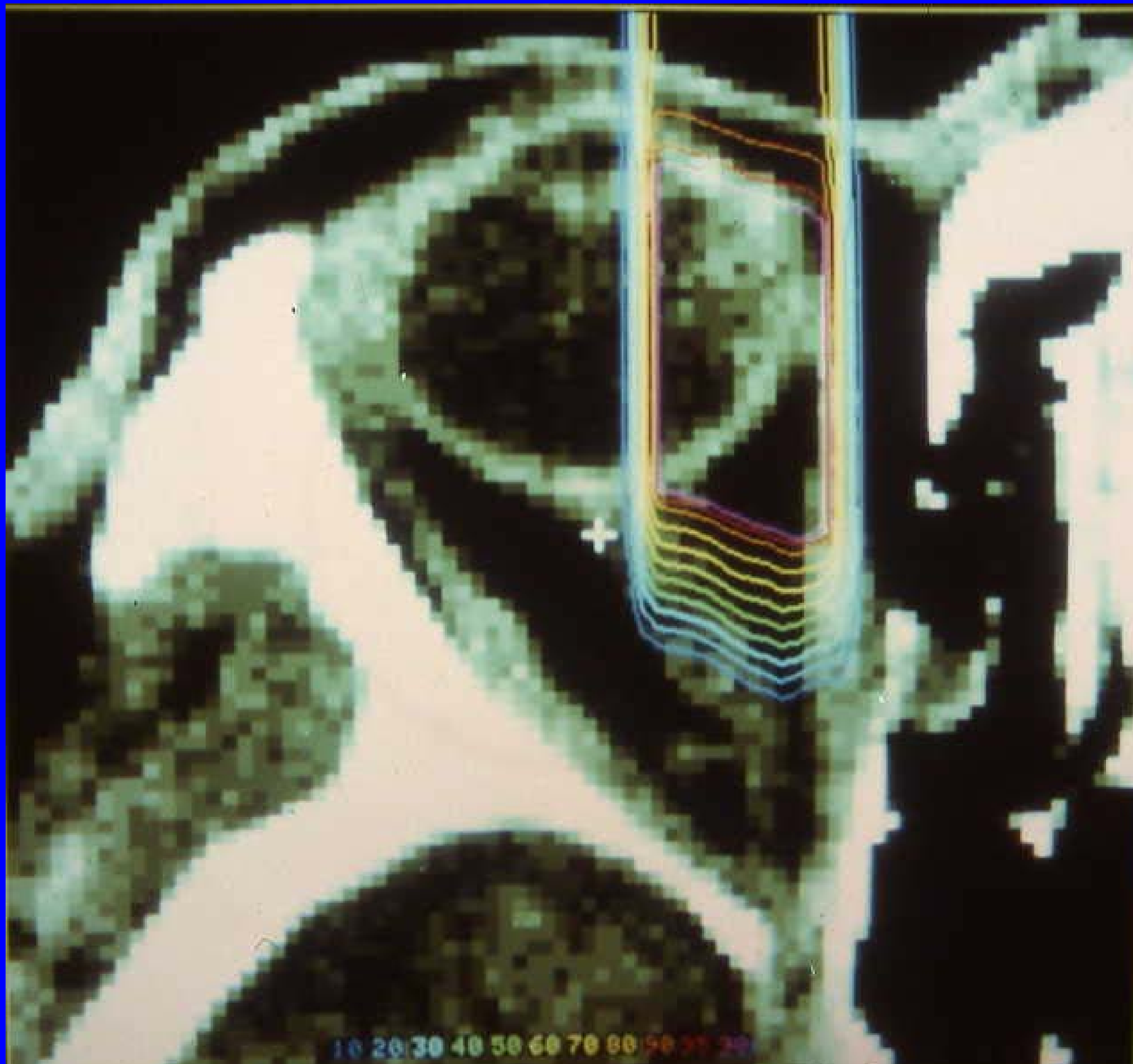
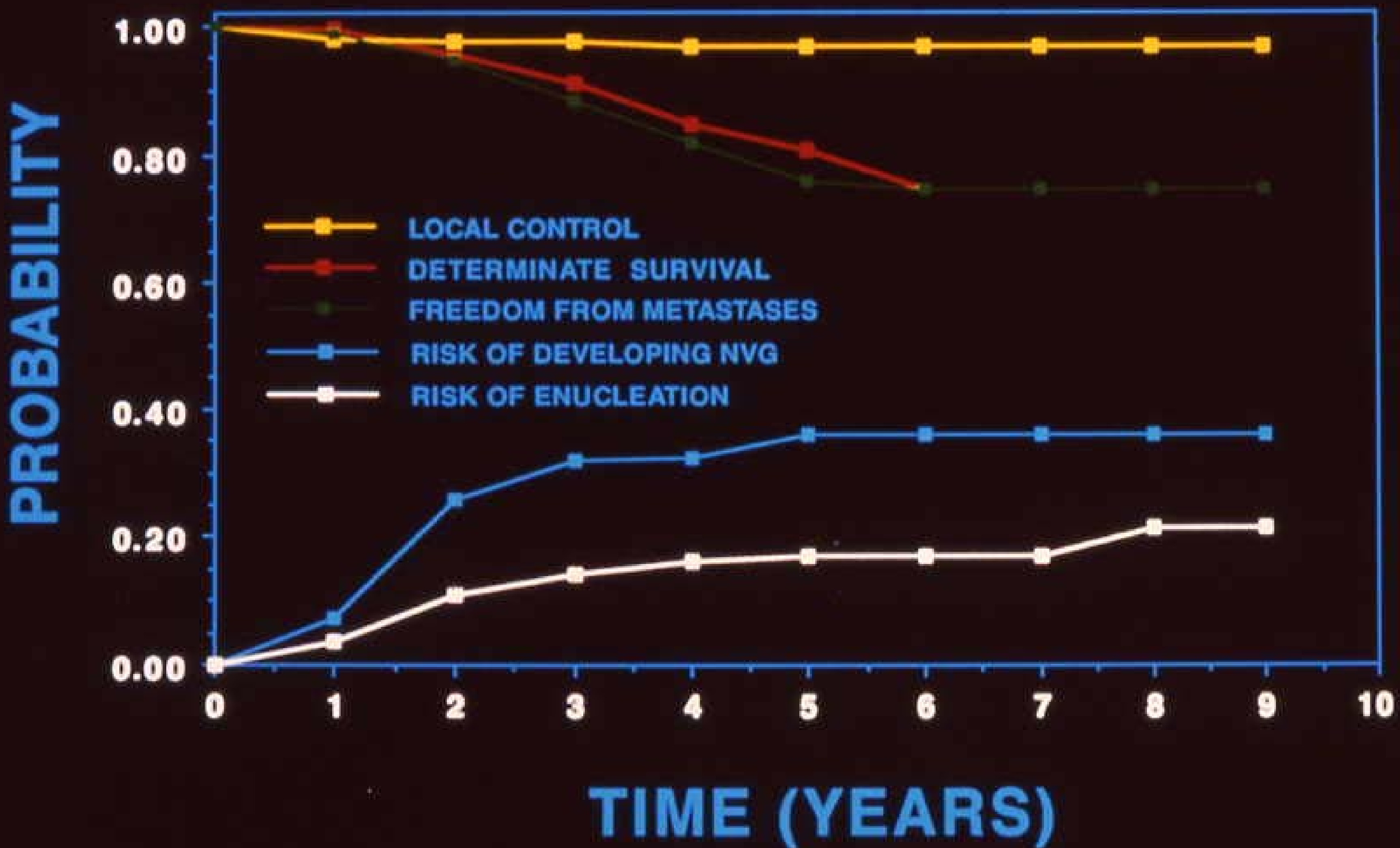


Fig. 3. Output from Massachusetts General Hospital treatment planning program.[7]

Int. J. Radiation Oncology Biol. Phys., Vol. 11, pp. 227–233


10 20 30 40 50 60 70 80

LBL HELIUM BEAM RESULTS: UVEAL MELANOMA



Radiation-induced cataract

- The human crystalline lens is known to be a radiosensitive tissue that responds with opacification in a delayed time course depending on the radiation type and exposure level.
- Opacification can be due to mal-folding of the crystalline proteins or due to misregulation of lens cell morphology.
- Cataracts are degenerative lesions that progressively increase, and can be defined in different ways, such as minor lesions not affecting sight, or as major lesions affecting vision.

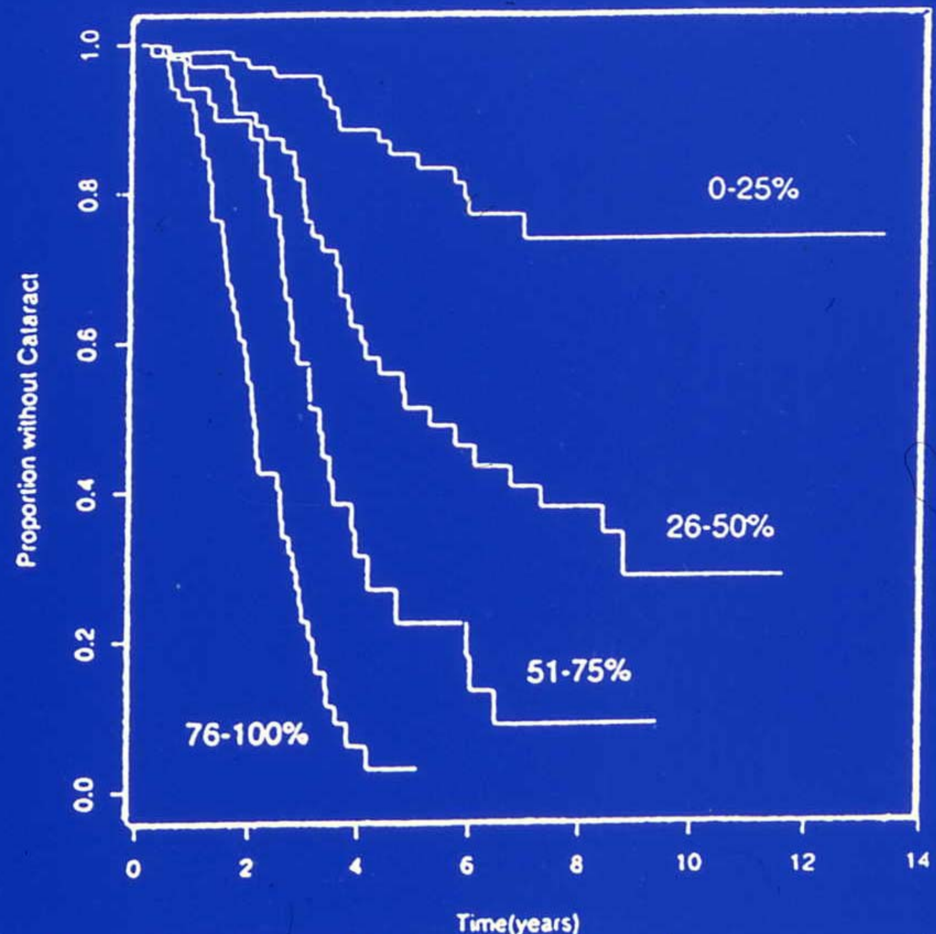
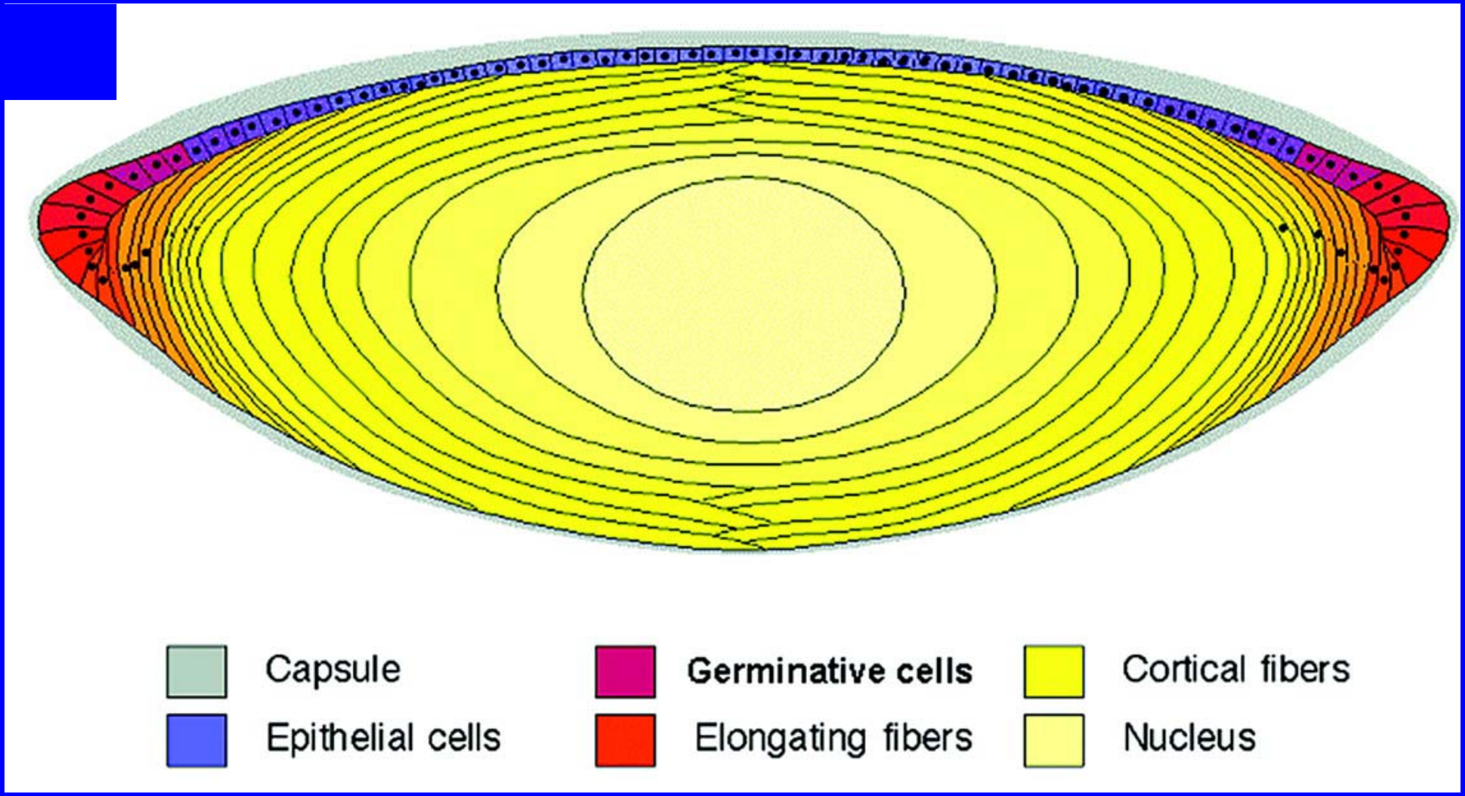


Fig. 6: Kaplan-Meier survival curves of cataract as a function of time after therapy, with the patient population anterior segment radiation exposure.(From Meecham et al., 1993).

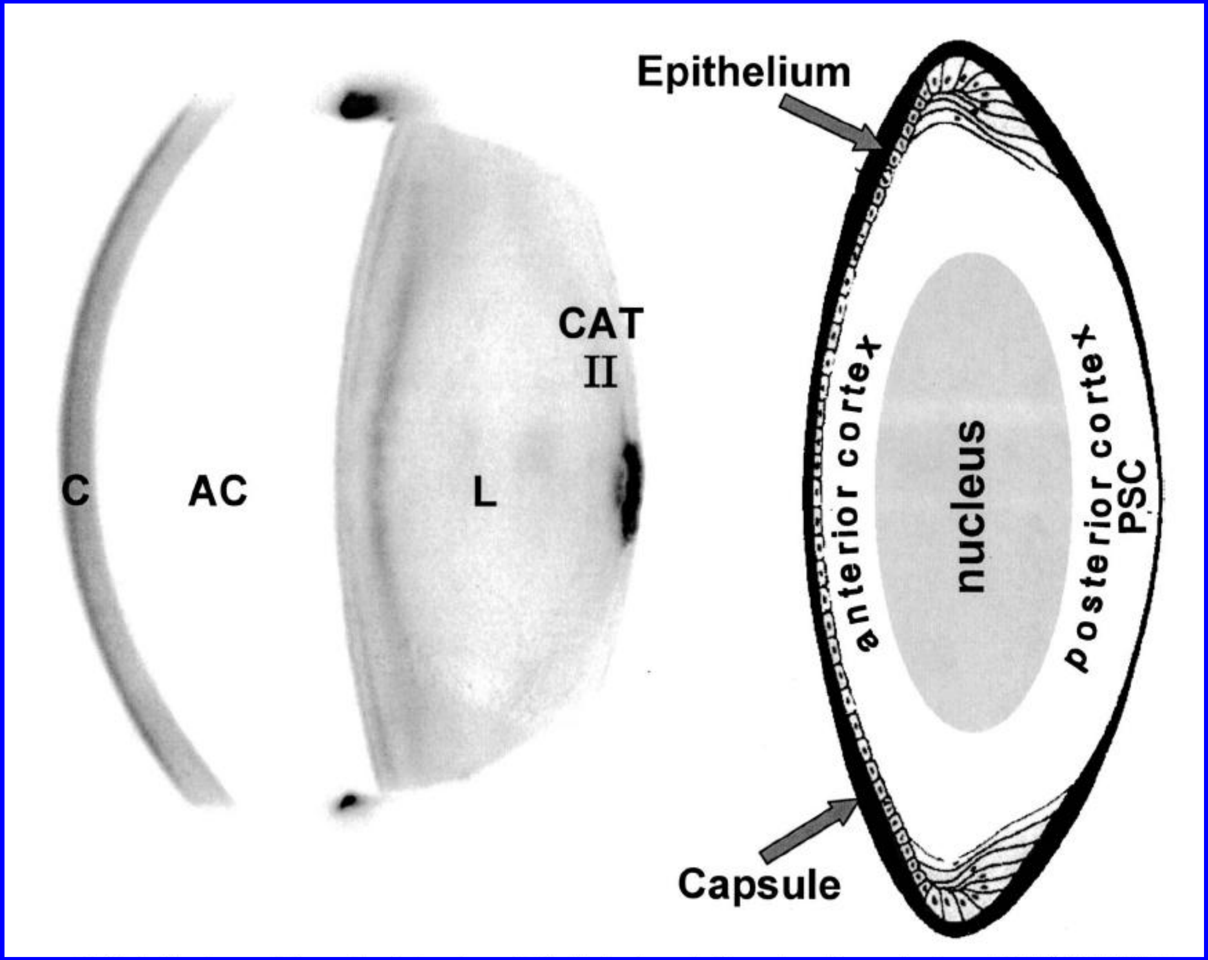
Cellular Organization of the Human Lens



Radiation-Induced Pre-Cataractous Cellular Changes in Human Lens

- Mitotic arrest of the germinative epithelial cells, followed by nuclear fragmentation & extrusion, and broadening of the nuclear bow with the appearance of abnormal mitoses
- Anterior cortical clefts appear & granular dots follow the line of fiber cells
- Abnormal fiber cell migration toward posterior pole of the lens
- Fiber cell swelling and interfibrillar clefts
- Appearance of multiple posterior subcapsular opacities due to the posterior displacement of abnormal epithelial cells
- PSC progresses in area as a granular white opacity

Cataract from a Chernobyl Clean-up Worker



Worgul et al., *Radiat. Res. 167*, 233, 2007

Age-Related Cataracts

- Nuclear Cataract
  - Causation linked to Smoking
- Cortical Cataract
  - Causation linked to diabetes & excess UV-B
- Posterior Subcapsular
  - Causation linked to steroids, diabetes, and IR
- Supranuclear
  - Causation linked to AD, Down’s Syndrome

Why do opacifications form in different anatomical locations in the lens?

- *Antioxidants are unevenly distributed*
- *Water diffusion system redistributes small molecules, etc.*
- *Regions of the lens have diverse signaling receptors*

Regional Distribution of Glutathione in Different Forms of Human Cataract

- Content of glutathione is high in the anterior lens cortex & epithelium, and in the posterior lens cortex & does not decrease with age
- Glutathione content is substantially lower in the lens nucleus and in supranuclear cataract
- The subcapsular cataract shows a rapid

  and pronounced progressive decrease in glutathione content

Pau et al., 1990

Radiation Cataract in Animal Models

- Cataract appearance after radiation exposure is dependent on:
  - –Radiation type
  - –Radiation dose
  - –Radiation fractionation
  - –Radiation dose-rate
  - –Animal species and genetic background
  - –Age and gender of animal at exposure
  - – Life-span of the animal
  - –Diet and presence of certain drugs

Problems with Radiation Cataract Studies in Animal Models

- Numerous cataract scoring systems have been used that cannot be easily normalized.

- Difficult to extrapolate time-course of radiation-induced human cataract from animal models with diverse life spans and genetic backgrounds

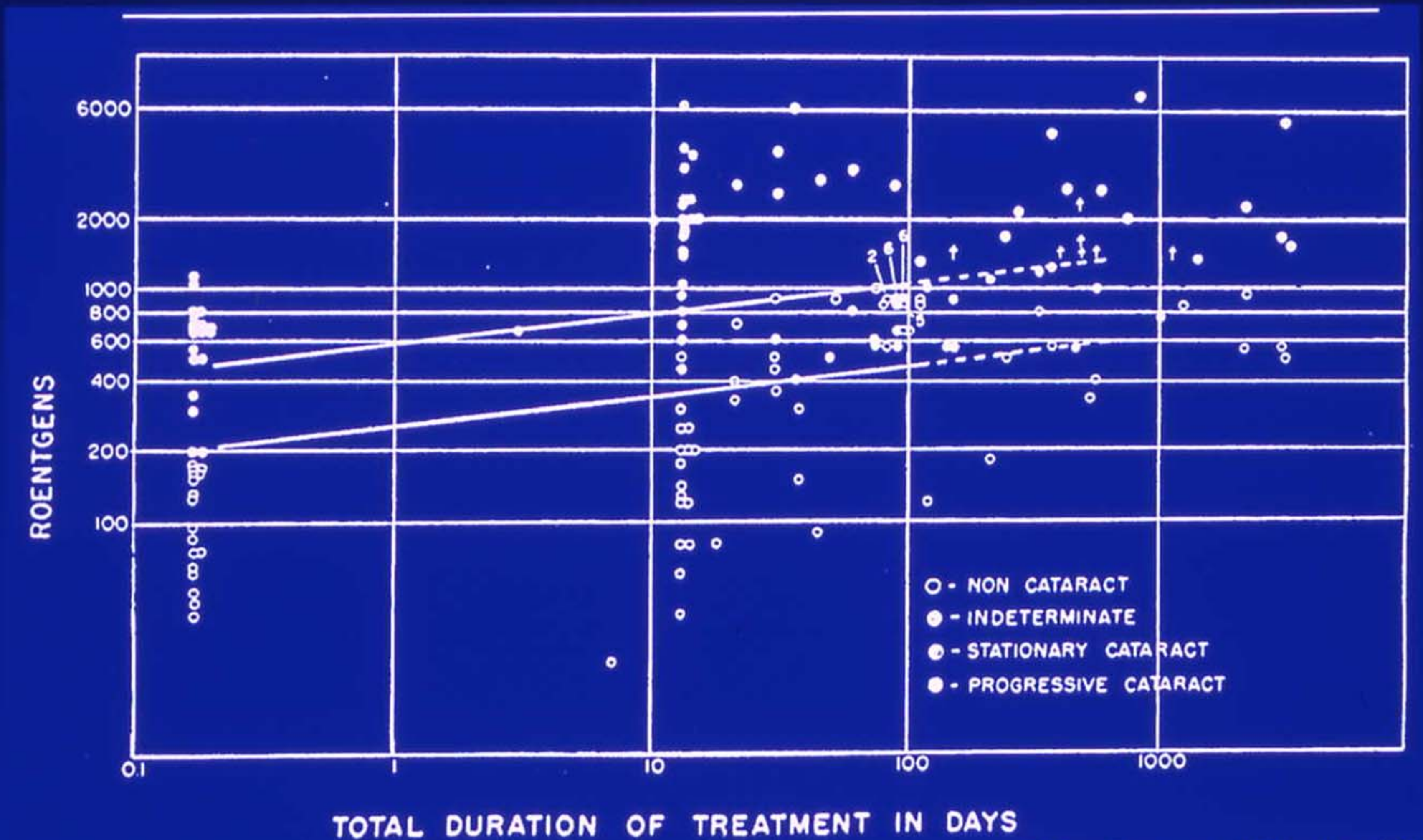
Radiation Cataract in Humans

- *Radiation accident victims*
- *Patients treated with radiation for disease or medical conditions*
- *Occupationally-exposed radiation workers*
- *Atomic Bomb Survivors*

Radiation Cataract in Humans Treated with RT for Cancer

- Opacification of transparent lens has been attributed to damage of the germinative epithelium resulting in a defective differentiation of lens fiber cells.
  - Clinical cataract incidence has been correlated with percent lens in the radiation field
- Review of RT case histories with lens exposure by Merriam & Focht in 60's indicated no opacities were observed with single acute doses of less than about 2 Gy, with the lens tolerating a higher dose with increased fractionation and overall treatment time.
- There is a dose-dependent latency in the appearance of the opacity after lens exposure, with higher doses showing cataract sooner.

Dose for Cataract/Non-Cataract Cases Plotted vs. Overall Treatment Time



Merriam and Focht, 1962

Radiation Cataractogenesis: A review of recent studies

Ainsbury EA, Bouffler, SD, Dorr W, Graw, J, Muirhead CR, Edwards, AA, and Cooper J

*Radiation Research 172:*1-9 (2009)

Conclusions

- Etiology of cataracts is not fully known, but is likely multifactorial.
- Much of the published evidence for radiation cataract at low dose is contradictory but pointing to little or no dose threshold.
- Not clear whether a mutational mechanism or one based on lens cell function, differentiation, cell killing and/or death is operating.

Conclusions from Cataract Studies of Exposed Individuals from Chernobyl Accident

- Linear-quadratic dose-response models yielded mostly linear associations with weak evidence for upward curvature
- The data do not support the ICRP 60 risk guideline assumptions of a 5-Gy threshold for “detectable opacities” from protracted, primarily low-LET, radiation exposures, but rather point to a dose-effect threshold of under 1 Gy.
- Thus, given that cataract is the dose-limiting ocular pathology in current eye risk guidelines, revision of the allowable exposure of the human visual system to ionizing radiation should be considered.

Worgul et al., *Radiat. Res. 167*, 233, 2007

RADIATION RESEARCH ***156***, 460-466 (2001)

Space Radiation and Cataracts in Astronauts

F.A. Cucinotta,[a] F.K. Manuel,[b] J. Jones,a G. Iszard,[b] J. Murrey,[c] B. Djojonegro[c] and M. Wear[c]

[a]NASA Johnson Space Center, [b]Kelsey-Seybold Clinic, and [c]Wyle Laboratories, Houston, TX 77058

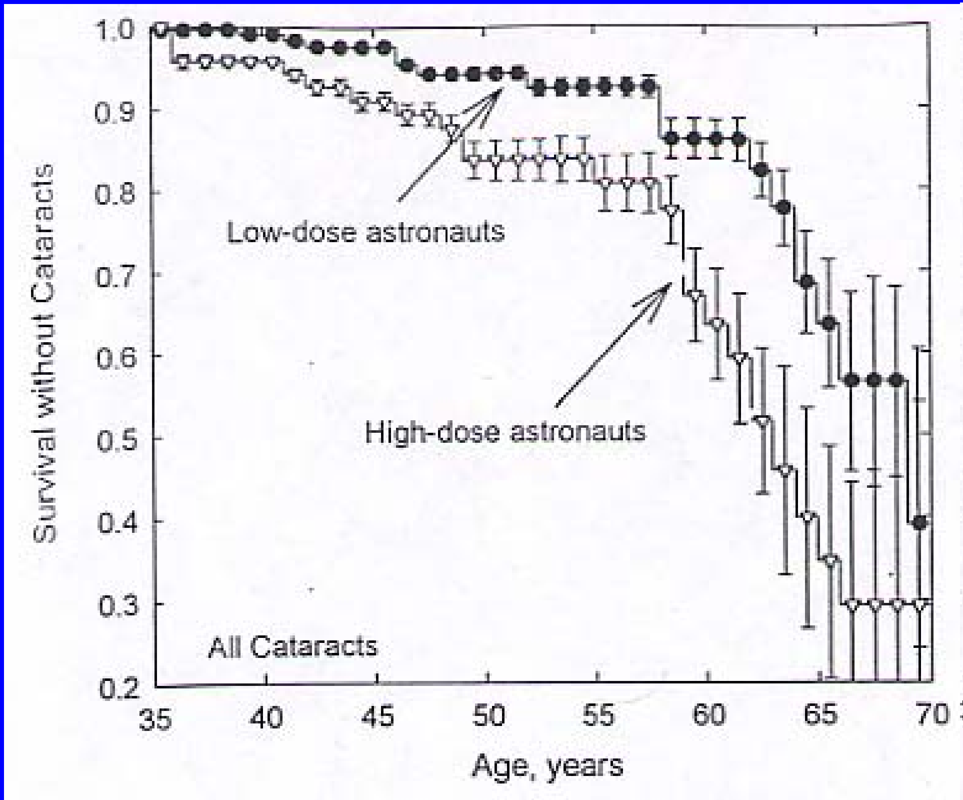
Conclusions from Particle Radiation Studies in Rodents

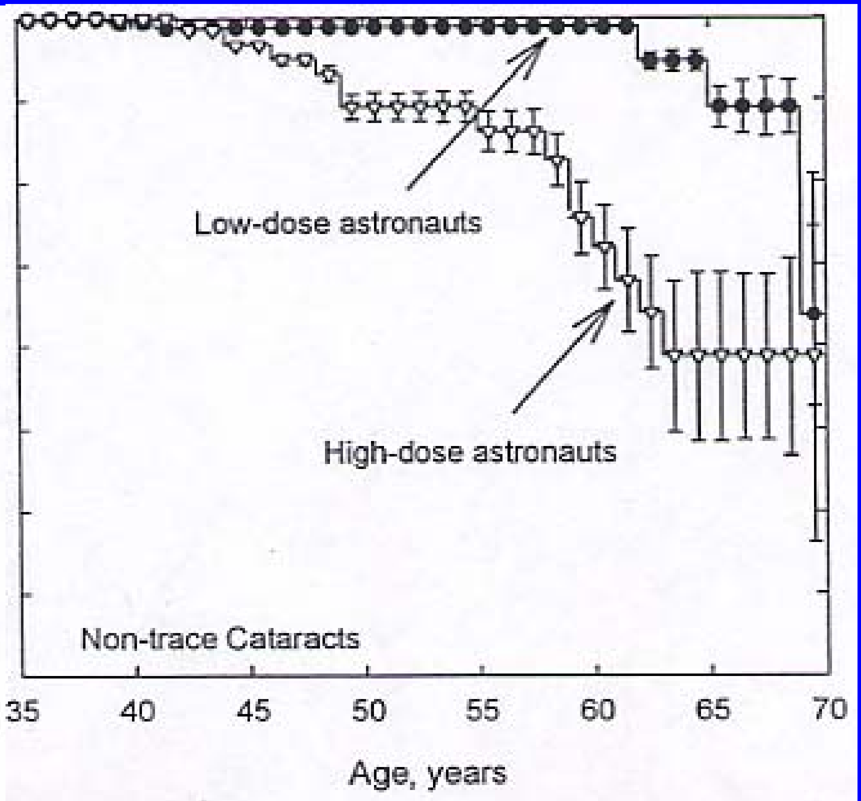
- Low particle fluences of HZE can cause cataract in WT strains with a high RBE (Worgul, Brenner)
- Particle dose-fractionation can enhance cataract induction (Worgul, Brenner)
- Radiation-sensitive mice (with DNA repair deficiencies) get HZE-induced cataract at lower doses and with shorter latency (Worgul, Hall, Kleiman).
- Particle-induced cataracts are gender-, hormone- and age-dependent (Dynlacht, Henderson)
- BBI can protect against particle induced cataract (Kennedy et al.)

Probability of Survival Without Cataracts as a Function of Age

Low-dose group:
Avg 3.6 mSv

High-dose groups:
Avg. 45 mSv





Cucinotta et al., 2001

Relative Hazard Ratios at Age 60 Comparing the High-Dose Group to the Low-Dose Group

Cataract type	Lens dose from all radiation sources	Lens dose from space radiation only
All	1.51 (0.64, 3.59)	**2.35 (1.01, 5.51)**
Non-trace	2.47 (0.76, 8.01)	**8.04 (2.51, 25.7)**
Cortical or dot	1.64 (0.51, 5.27)	1.44 (0.46, 4.65)
Nuclear	0.83 (0.18, 3.81)	3.47 (0.79, 15.3)
PSC	1.1 (0.67, 18.1)	5.76 (0.97, 34.2)
PSC, Nuc or Mixed	1.33 (0.37, 4.83)	**3.73 (1.05, 13.3)**

Cucinotta et al., 2001

NASA Study of Cataract in Astronauts (NASCA). Report 1: Cross-Sectional Study of the Relationship of Exposure to Space Radiation and Risk of Lens Opacity

Chylack LT, Peterson LE, Feiveson AH, Wear ML, Manuel FK, Tung WH, Hardy DS, Marak LJ, and Cucinotta FA

Radiation Research 172, 10-20 (2009)

Conclusions (Chylack et al., 2009)

-Cross-sectional data for astronauts & matched ground control subjects were analyzed by fitting customized non-normal regression models to examine the effect of space radiation on nuclear, cortical and PSC opacities.

-GCR may be linked to increased PSC area and the number of PSC centers.

-Within the astronaut group, PSC size was greater in subjects with higher space radiation dose.

Conclusions (Chylack et al., 2009)

-No association was found between space radiation and nuclear cataracts.

-Cross-sectional analysis revealed a small deleterious effect of space radiation for cortical cataracts and possibly for PSC cataracts

-These results suggest increased cataract risks at smaller radiation doses than have been reported previously

Rationale

- Radiation can cause cataract.
- There is a dose-dependent latency after radiation exposure before cataract appears.
- At low doses the latency is longer.
- It has been assumed that not much happens during this latency period.
- We are studying molecular antecedents to frank particle-induced cataract during the latency period to identify molecular markers early enough to allow biological countermeasures to be devised.

HYPOTHESES

for mechanism of radiation cataractogenesis

- Increased genotoxic load of damage leads to cataract through a number of intermediate steps leading to altered gene expression

- Gene expression is altered without genomic changes at the level of signaling

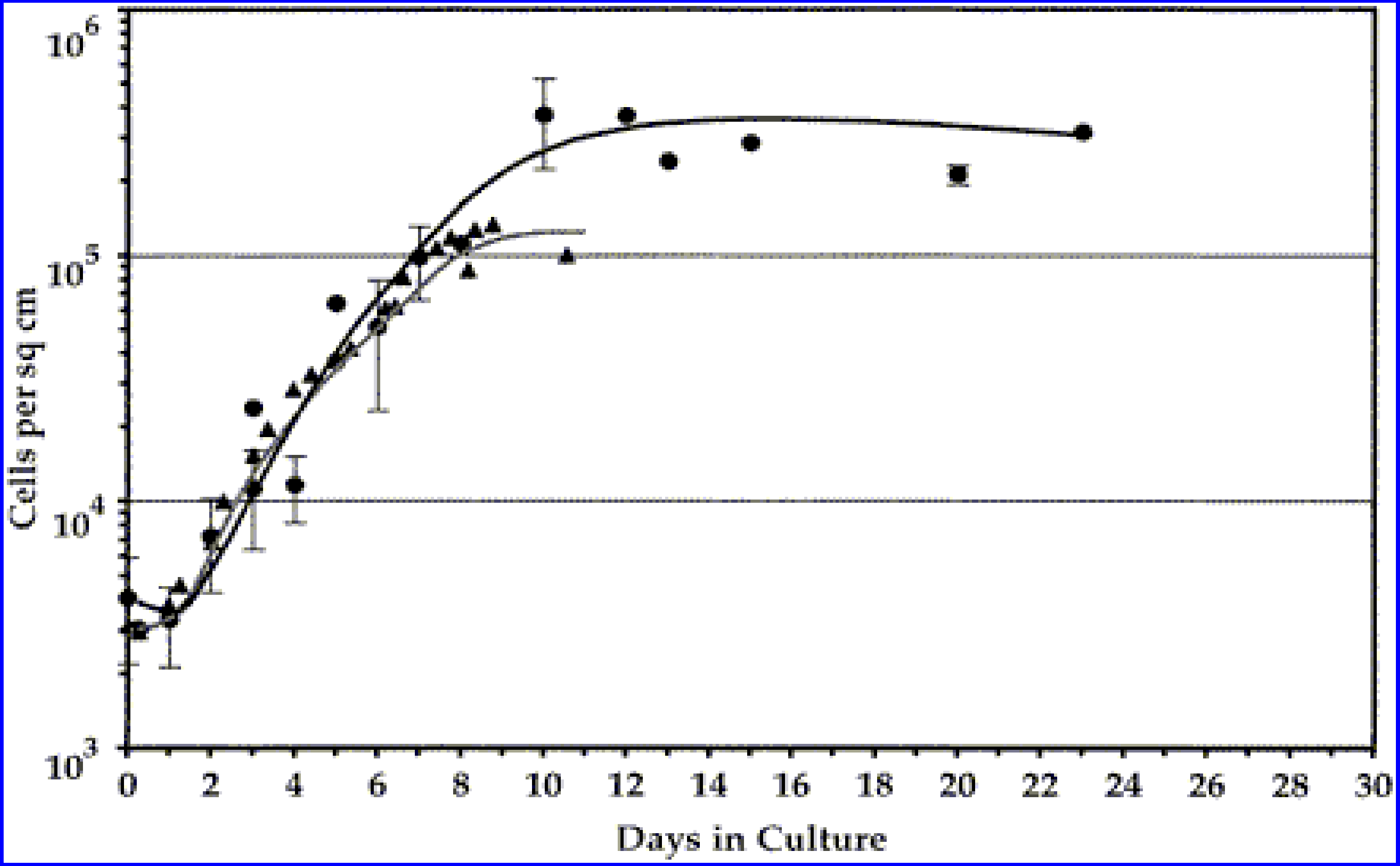
- The effect is on protein expression directly

- There is the possibility that these three hypotheses are not mutually exclusive, and that some combination is involved

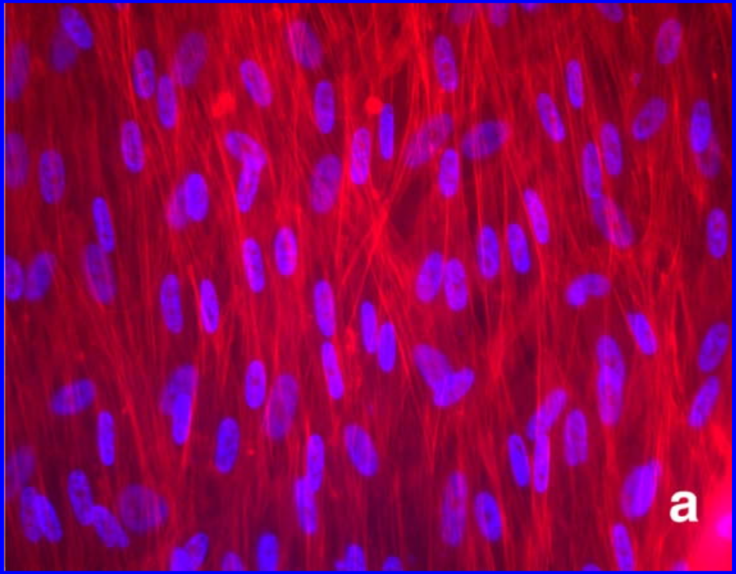
Technical Approaches

- Lens cell differentiation *in vitro*
- Lens cell changes in mice *in vivo*
- Molecular biology of genes and proteins underlying radiation response
- Molecular effects leading to protein aggregation

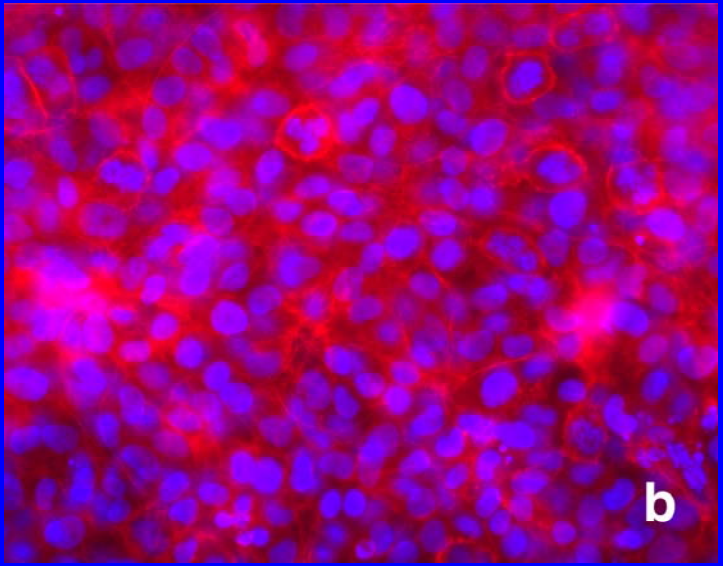
Growth kinetics of Non-transformed HLE & Virally-transformed HLE B-3 cells



Actin Filaments in Confluent Lens Cultures

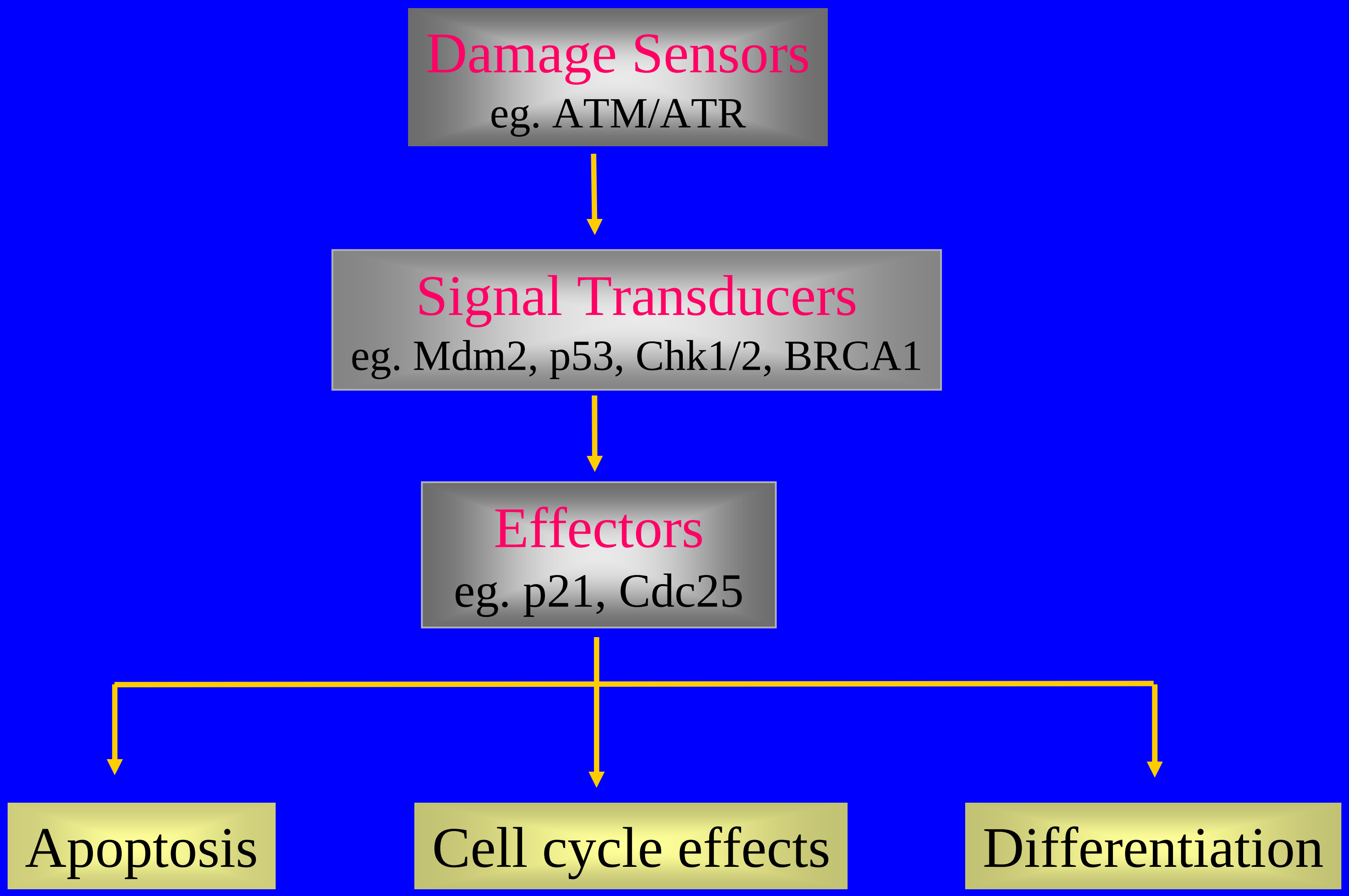


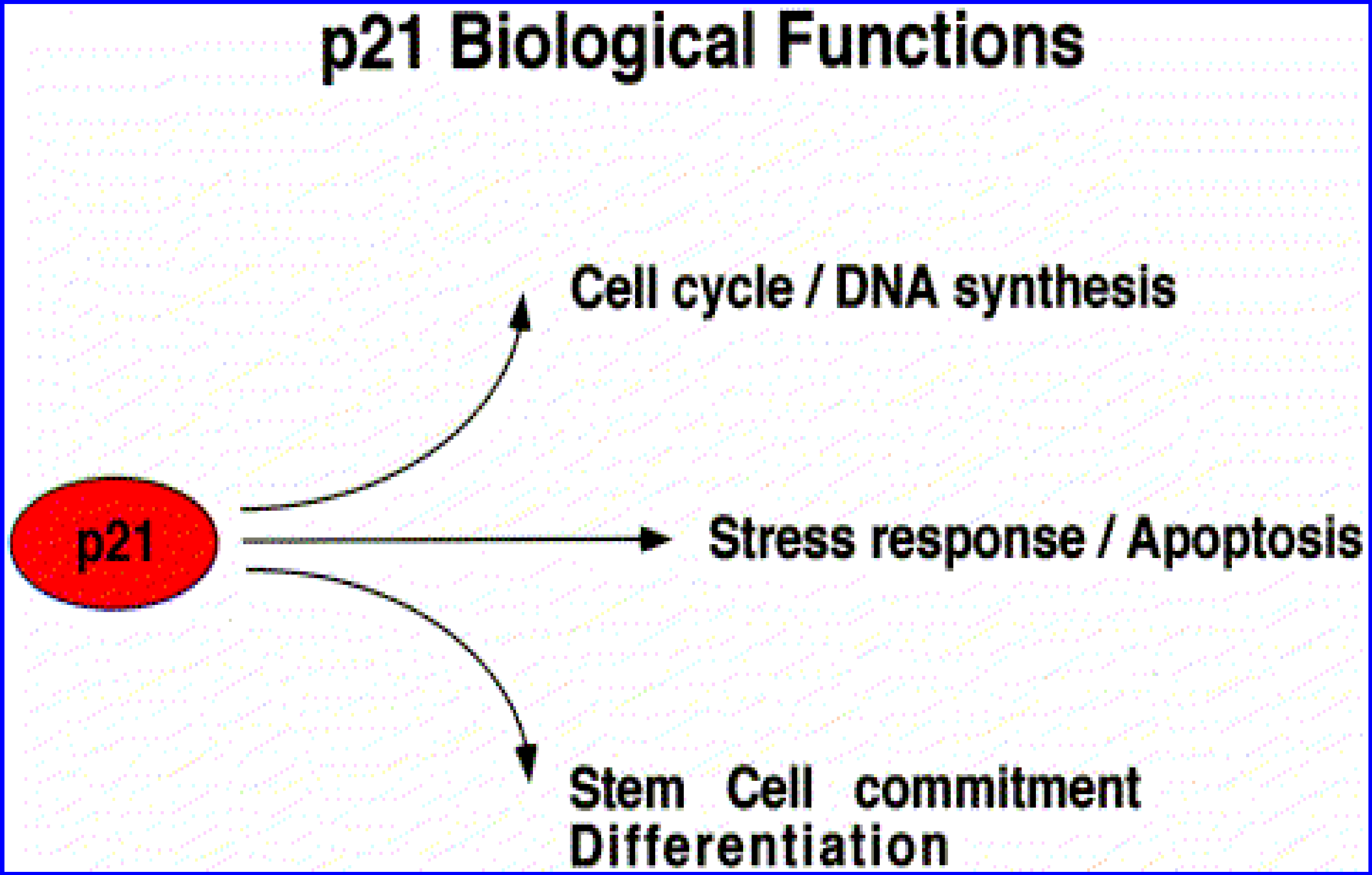
Nonimmortalized HLE Cells



Transformed HLE B3

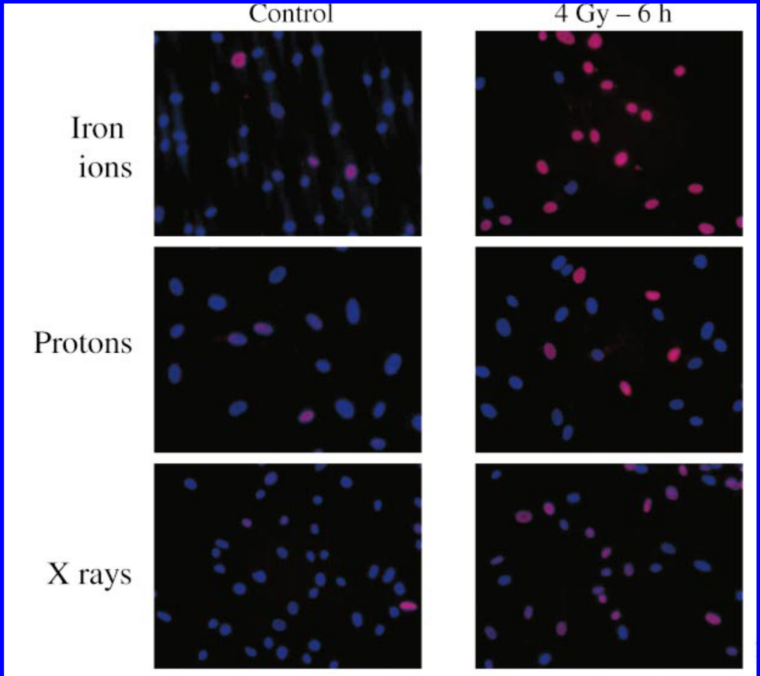
Ionizing Radiation-Induced Damage

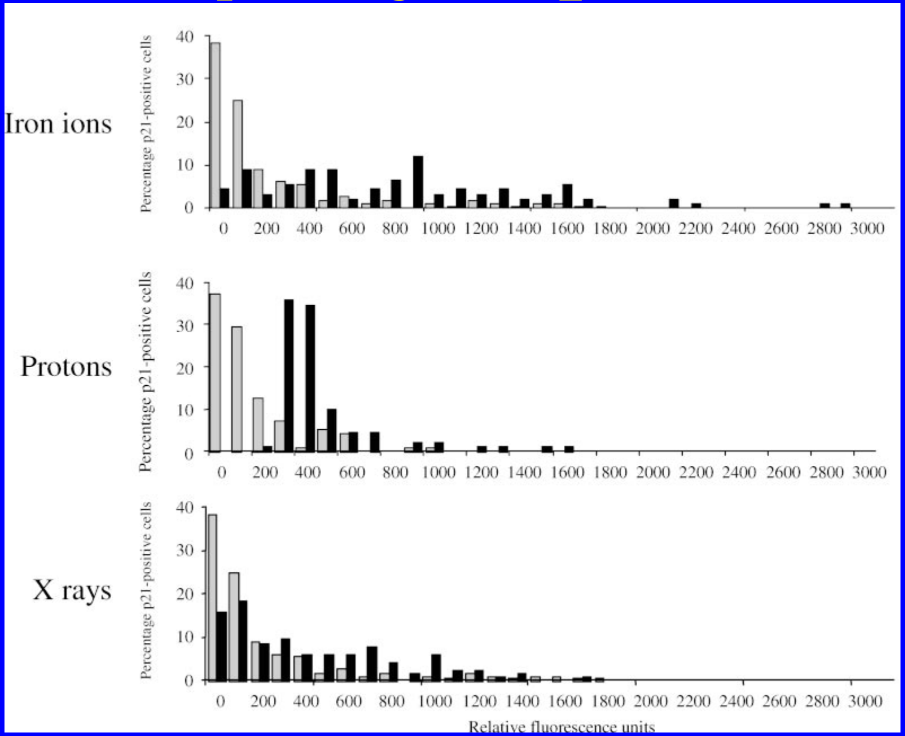




G.P. Dotto
Biochem. Biophys. Acta, 1471: M43 (2000)

CDKN1A protein expression in human lens epithelial cells is radiation-quality responsive

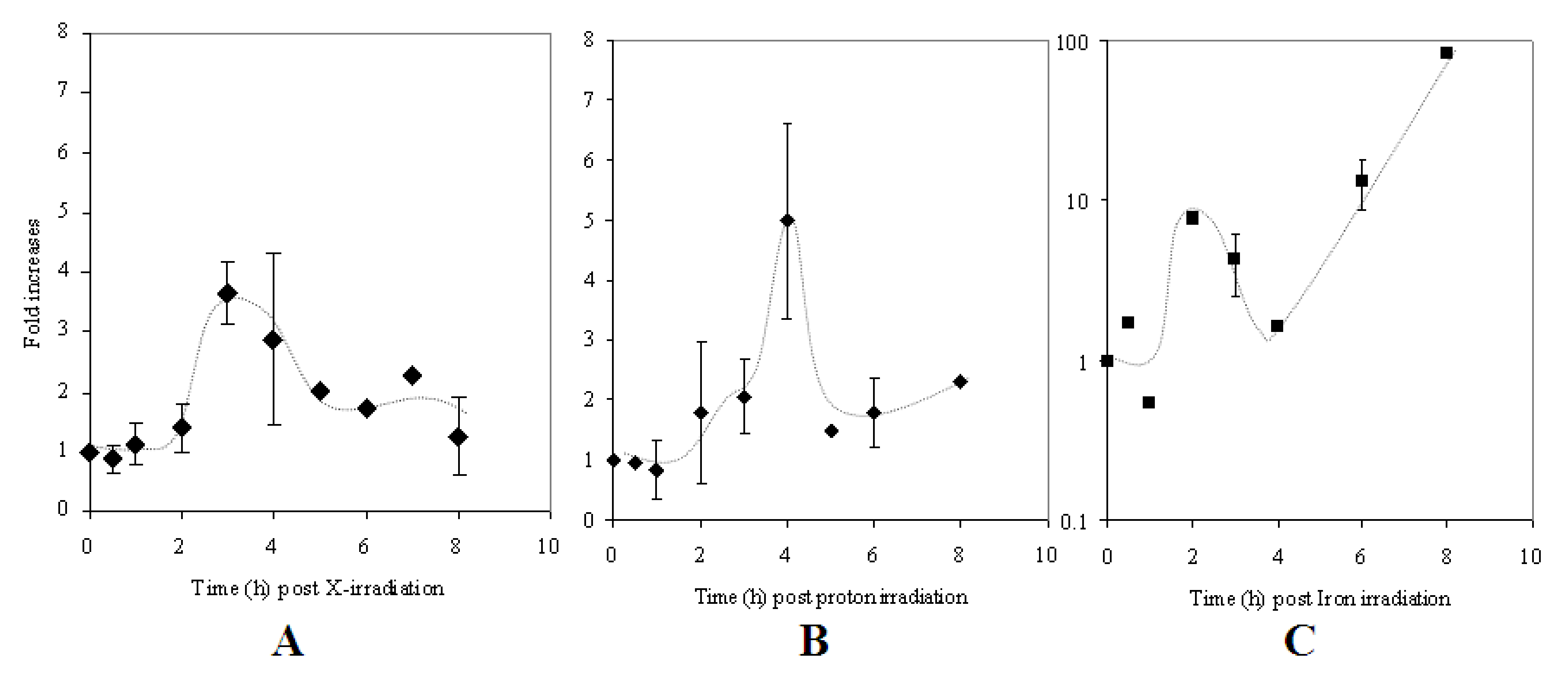




- A high percentage of CDKN1A-positive cells in the respective control samples (gray bars) show relatively low fluorescence levels.
- The percentage of CDKN1A-positive cells in the 4-Gy-irradiated samples (black bars) is shifted toward higher fluorescence intensities.

Chang et al, Radiat.Res. 2005

Kinetics of CDKN1A gene expression in human lens epithelial cells is dependent on radiation quality



- The relative expression for panels A and B is presented in linear units.
- To fully represent the magnitude of up-regulation of CDKN1A transcripts after exposure to iron ions, the relative expression for panel C is presented in log units.
- The line connecting the data points represents a temporal trend suggested by the distribution of data points.

Chang et al, Radiat. Res. 2005

Screening for radiation-induced functional genes

Microarray Gene Family	Radiation-responsive genes
Cell cycle genes	p21; Cyclin G1; cdc2 (cdk1); Protein kinase Chk2; MCM6
TP53 gene family	TRAF & TNF-receptor associate protein, Apoptotic protease activating factor, APEX nuclease; BBC3 (Bcl-2 binding component 3); BRCA1; CDC2; CDNK1A (p21); CHEK1, p16, TP53BP2; WIG1 (p53 zinc finger protein); TNF alpha-induced protein.
Extracellular Matrix genes	Cathepsin B and D; Integrin alpha 5; matrix Metalloproteinase 1; Plasminogen activator urokinase, E-Cadherin, Thrombospondin-3 gene
Stem cell genes	CTNNB1(Catenin beta1); CDKN1A (p21); CD9 (p24); FGF2 (basic);HSPA9B(hsp70kD protein 9B):IGF2R (insulin growth factor 2 receptor); IL6ST (Interleukin 6 signal transducer= gp130);ITGAV (integrin alpha V); MDM2; NCAM2.

Evidence for radiation-induced premature and defective differentiation

- Morphological
  - Premature fiber cell elongation & alignment
  - Abnormal fiber cell alignment
  - Lack of complete enucleation
- Functional
  - Premature appearance of fiber cell markers including,
    - Cell adhesion molecules (b1-integrin, a5 integrin, a6B to a6A isoform switching)
    - Connexin 50
    - MMPs changes in expression

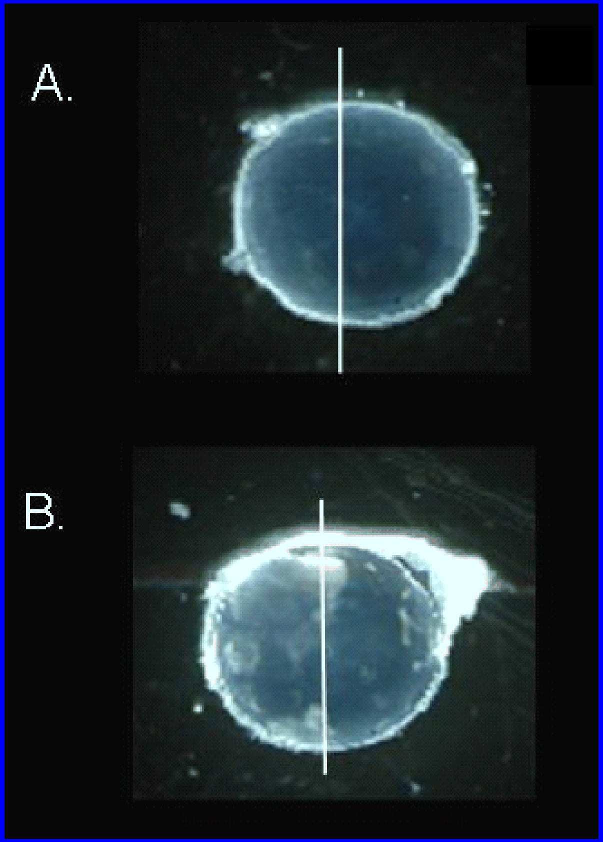
In vivo confirmation

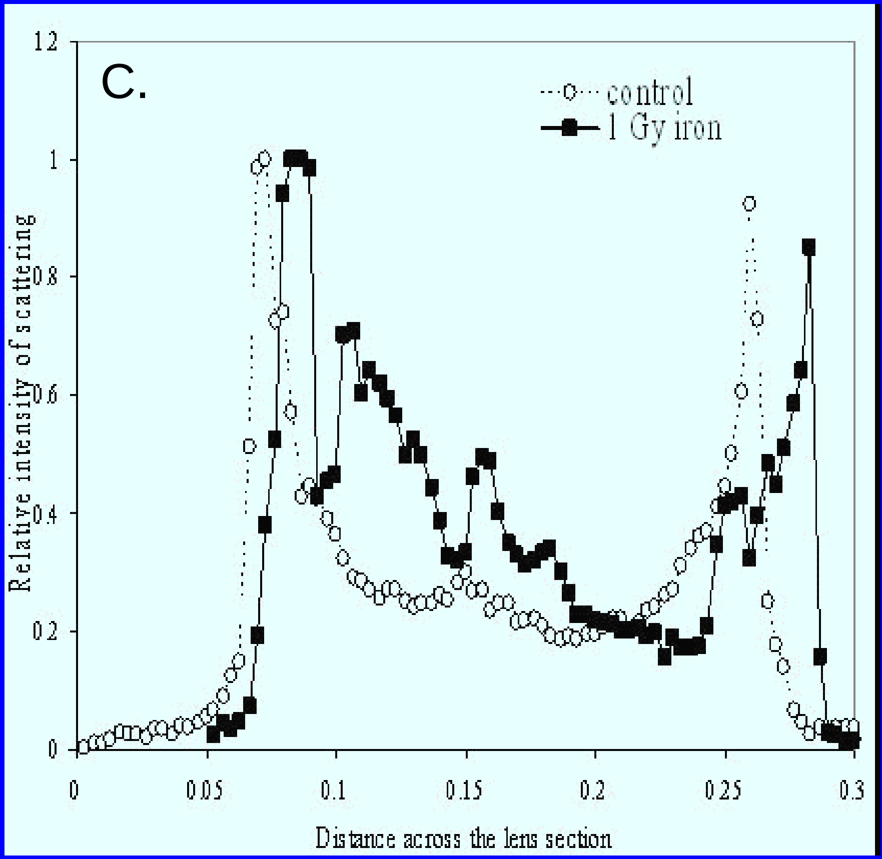
- Sprague-Dawley rats were irradiated in a pilot study in NSRL-2 with high energy iron or titanium ions
- Goal to complete immunohistochemistry of early marker proteins on nascent particle-induced Sprague-Dawley cataracts to correlate with molecular changes in human lens data *in vitro*

Cataract Scoring

- The lenses of these animals were examined by a veterinarian ophthalmologist with a slit-lamp examination while the pupils were fully dilated at monthly intervals over a period of nine months.
- At the time of terminal necropsy, photographic images of the lenses were obtained *ex vivo*.
- The harvested lenses were either frozen without fixation for mRNA and protein analyses, or fixed with PFA for immunohistochemical analyses with specific probes.

Ex vivo lens imaging

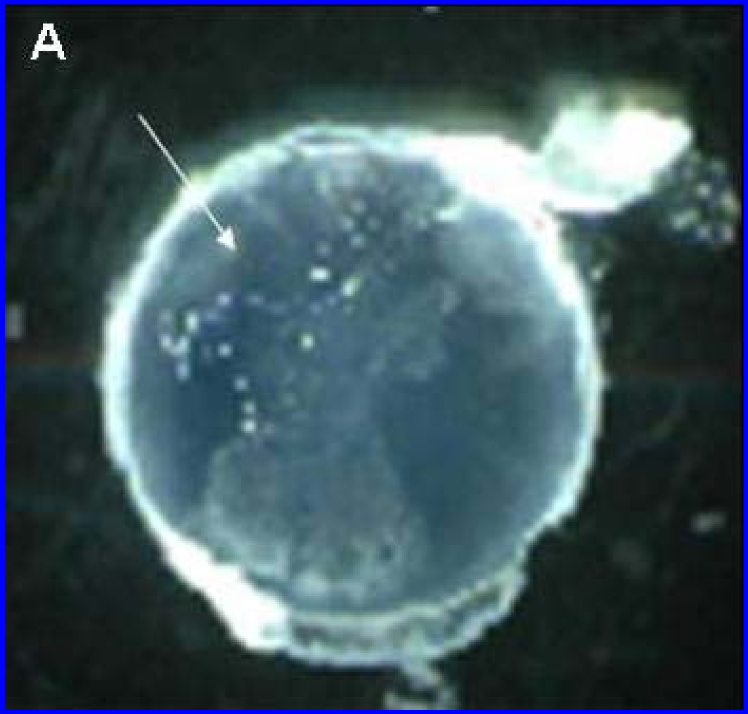




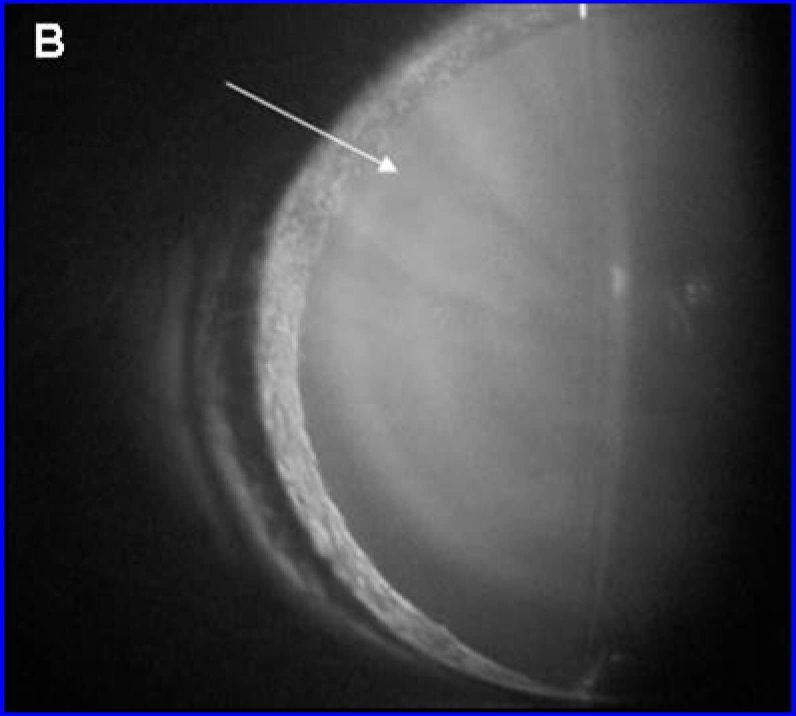
(A) *Ex vivo* image of a lens from an unirradiated control animal. (B) *Ex vivo* image of a lens from an animal with anterior cortical cataract in the 100 cGy dose group.

(C) The densitometric analysis of the opacification of the lens using NIH Image J program illustrates relative differences in light units along the axis of analysis between the 2 lenses.

Ex vivo and in vivo lens imaging



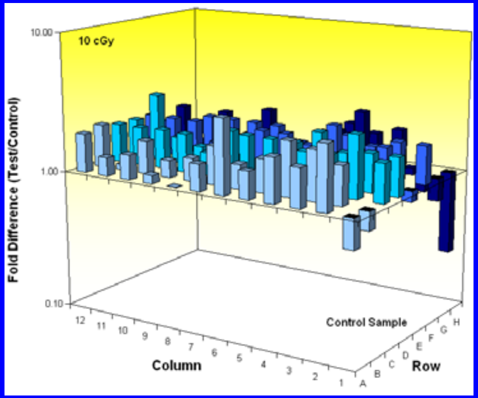
(A) *Ex vivo* image showing PSC dots in a representative lens photograph taken from a 100 cGy irradiated animal at 9 months after 600 MeV/amu iron ions.



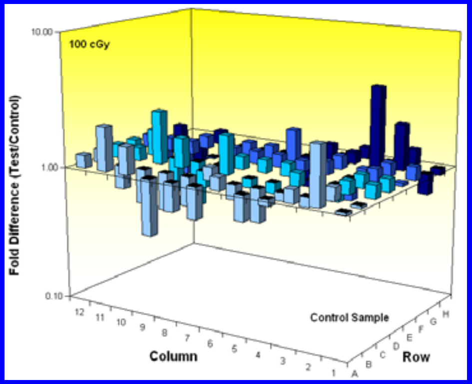
(B) Representative clip from video recording showing spokes and annular rings.

ECM and Adhesion Molecules Gene Expression Profiling

- Three-dimensional plots of the 96 gene Rat Extracellular Matrix and Adhesion Molecules Profiler Array gene expression analysis of 10 cGy and 100 cGy 600 MeV/amu iron-ion irradiated lenses normalized to unirradiated sham-treated control lenses.
- Each analysis included mRNA of lenses from 4 animals harvested 9 months after the irradiation.
- The high responsiveness of ECM gene families demonstrated after a low radiation dose to the lens *in vivo* contrasts with the decreased gene expression seen after a 10-fold higher radiation dose nine months after the exposures.
- Different genes are affected at each dose level.
- It is not yet known how this gene profile correlates with the status of the opacifications observed at each dose at the same time post-exposure.



10 cGy



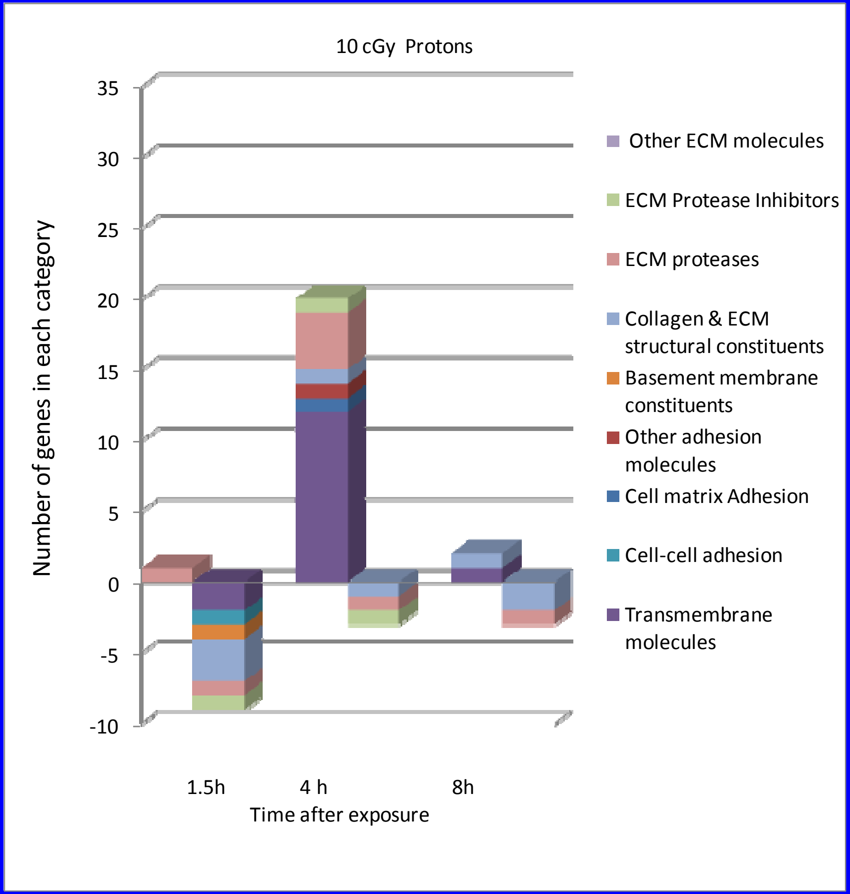
100 cGy

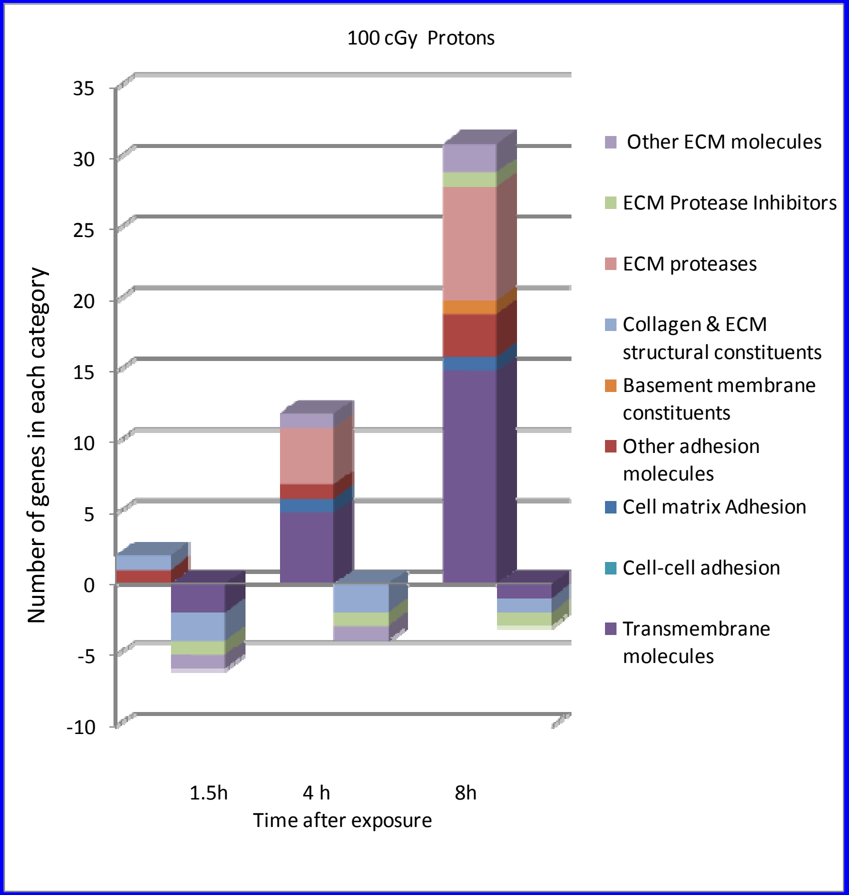
Summary of Gene Expression Profiles after Iron ions

Iron Dose (cGy)	**Gene name**	**Fold change**	**p value**	**Functional grouping**
10	CD44 antigen ***	3.43	0.095	CAM - transmembrane
	Procollagen, type IV, alpha 2 - Col4α2 predicted	2.46	0.06	ECM- basement membrane
	Extracellular matrix protein 1 - Ecm1	2.03	0.003	ECM- others
	Ectonucleoside triphosphate diphosphohydrolase 1	2.78	0.05	ECM- basement membrane
	Integrin, alpha 2 - Itgα2	2.24	0.075	CAM-transmembrane, cell matrix
	Matrix metallopeptidase 12 -MMP12	2.42	0.024	ECM protease
	TGFβ1	2.57	0.0575	ECM and CAM - others
100	A disintegrin-like and metallopeptidase with thrombospondin type 1 motif, 5 (aggrecanase-2) - Adamts5	2.71	0.075	ECM protease
	Cadherin 3, type 1, P-cadherin (placental) - Cdh3	-2.64	0.0064	CAM transmembrane
	Integrin alpha 5 - Igtα5	2.49	0.0518	CAM: cell-matrix adhesion
	Laminin, alpha 2 (predicted) - Lamα2	-2.14	0.0715	CAM-other adhesion molecule

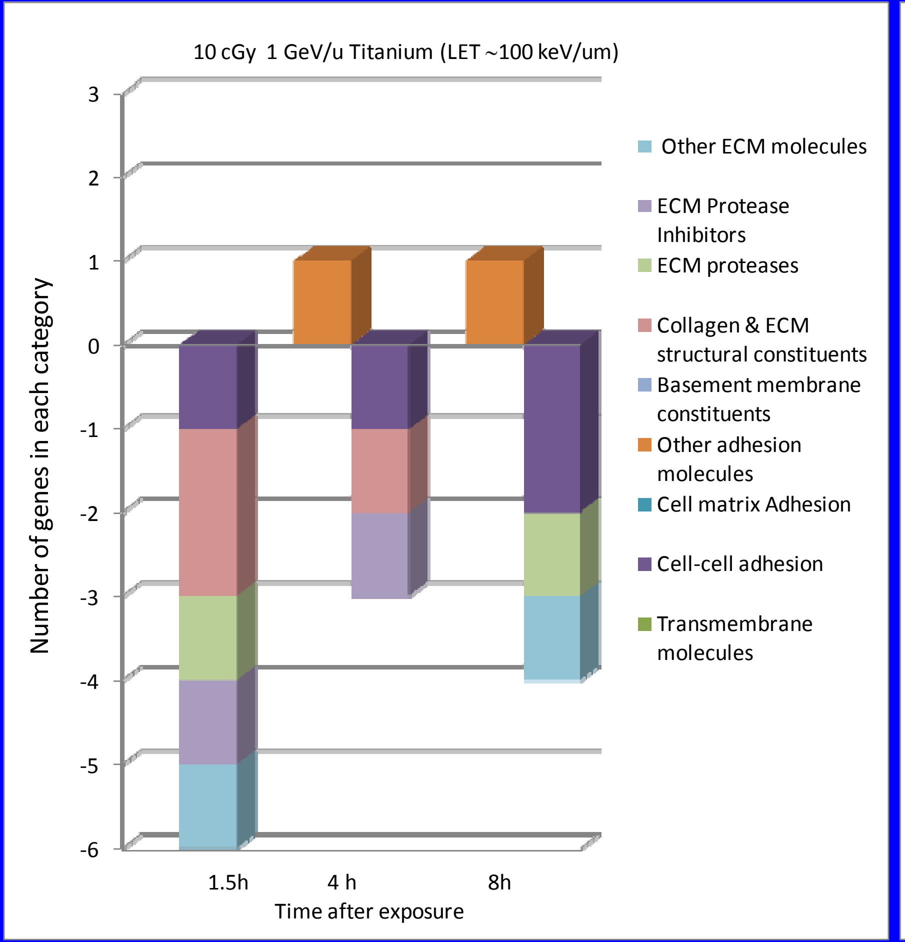
Note: CD44 was detected in Lens epithelial cells of human cataracts (IOVS 38, 579, 1997)

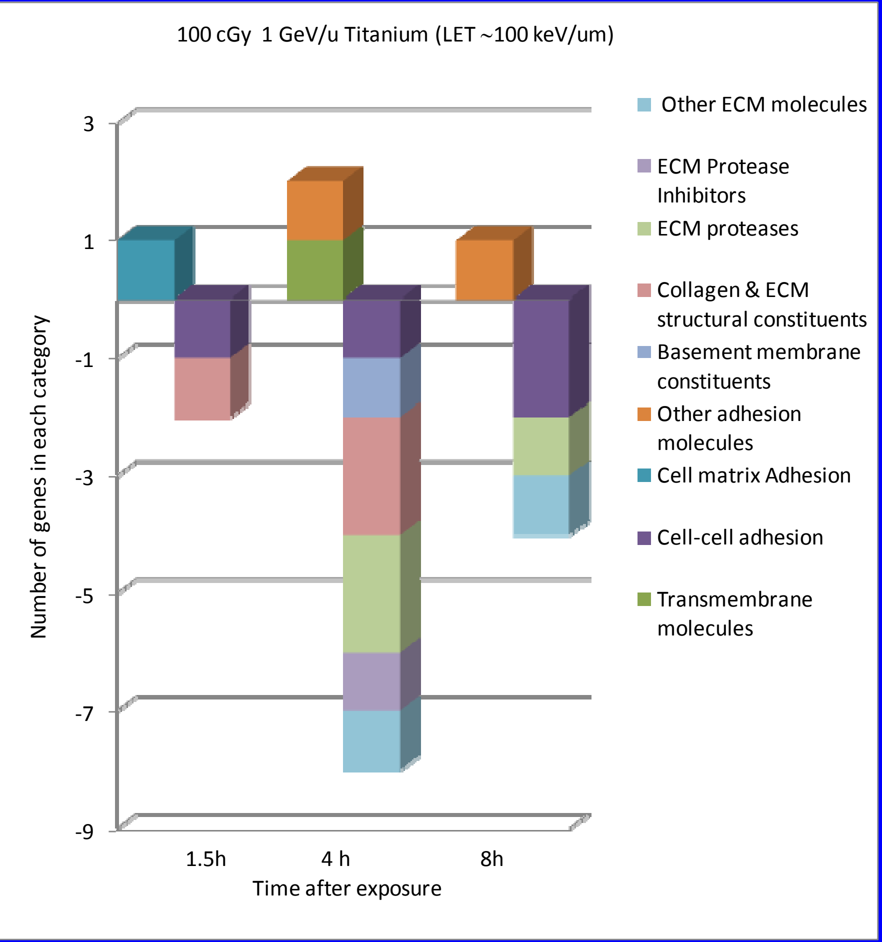
Dose and time dependent changes in expression of Extracellular Matrix proteins after Proton exposure



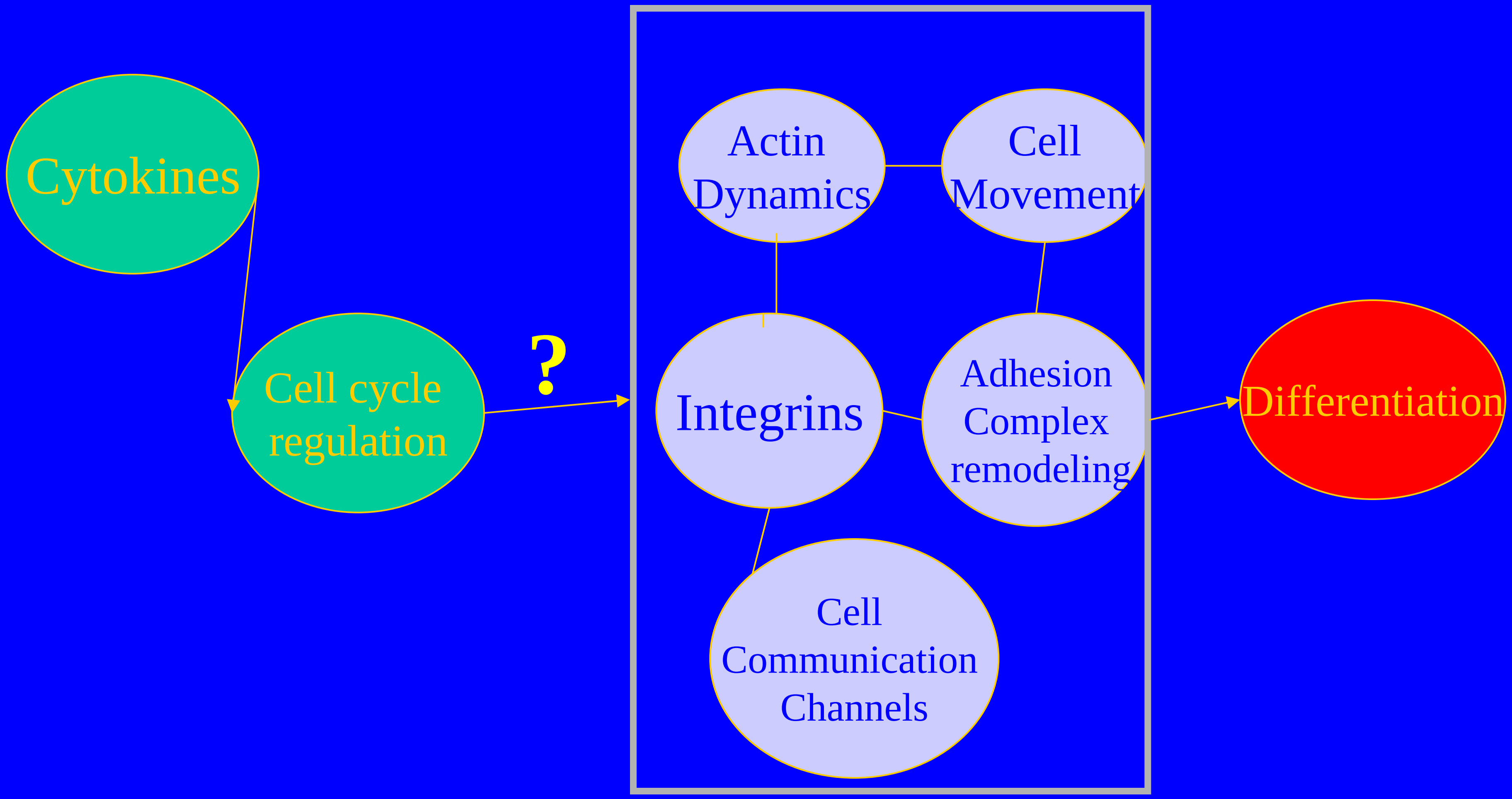


Dose and time dependent changes in expression of Extracellular Matrix proteins after titanium ion exposure





Lens fiber cell differentiation involves several radiation-responsive signal transduction pathways

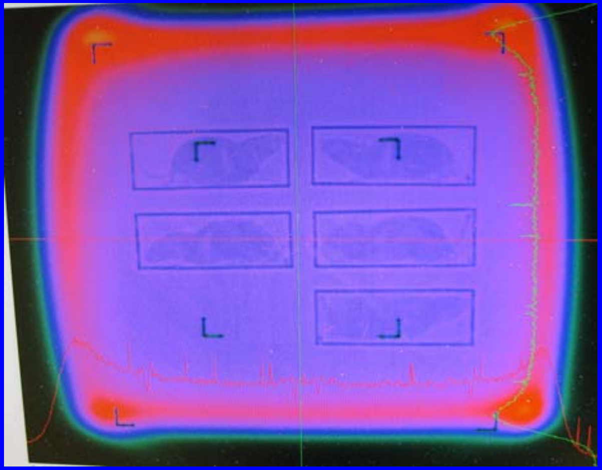


Hypothesis: Early molecular markers of radiation cataractogenesis will lead us to underlying mechanisms and countermeasures.

NSRL 08C 1 GeV Proton Irradiations

October 2008

Unanesthesized mice in their condo
On the Beamline Prior to Irradiation



Computer Screen
Showing Dose Uniformity,
and Position of Mice

Summary

- Data indicate radiation-quality- and dose-dependent differences in expression of ECM genes in epithelial cells
- Effects are dependent on differentiation status and may impact selective ECM remodeling leading to tissue pathology
- Changes in MMP-2 active and latent protein levels are noted during normal differentiation & are perturbed after heavy particle radiation
- *In vivo* studies are in progress to confirm anatomical development of cataract & role of specific gene products in cataract development

What makes particle radiation so effective?

- Track structure
- Clustered damage
- Production of short DNA fragments
- Slower repair
- Evidence of misrepair
- Genomic instabilities
- Microenvironmental changes
- LET-dependent gene responses

Some Unknown Factors in Assessing Light- and Heavy-Ion Damage

- Consequences of persistent chromosomal rearrangements
- Enhanced role in inducing genomic instability
- Significance of individual genetic susceptibility
- Late effects on tissues at low particle fluences and rates
- Induction of remodeling of basement membrane in ECM of tissues
- Importance of enhanced sensitivity of neural behavior
- Combined effects of multiple stressors

Why is it important to identify molecular pathways of action?

- We have the tools to understand the molecular pathology of cancer and how to use this information to treat individual cancers. (Harris & McCormick, *Nature Reviews Clinical Oncol*, 2010; Riedel et al., *Mol Cancer Ther*, 2008)
- Unique gene expression pathways are being reported in the literature for human tumors irradiated with radiations of different radiation qualities (Maalouf et al., *IJROBP*, 2009; Hamada et al., *Radiotherapy Oncology*, 2008; Higo et al., *IJROBP*, 2006)

New Era for Charged Particle Radiobiology

- Human genome mapped & being mined for tumor and normal tissue data on radioresponse
- Powerful new genomic & proteomic tools available
- Focus on individualized medicine
- Networks of gene & protein pathways identified
- Gene expression profiles change in a dose- and time-dependent fashion after exposure to particles of variable LET
- Tailored 3-D image-guided & intensity modulated physics
- Theoretical biophysical modeling is guiding treatment optimization, but more work is needed to understand microdosimetric energy deposition effects

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