Question: "What is the biological effect of a single alpha particle?"

Microbeams

Delivery of single or precise number of charged particles to individual cells.

Accuracy allows targeting of nucleus versus cytoplasm.

Avoid the Poisson distribution statistical problem.

Only a few microbeam facilities in the world
- Columbia University
- Gray Laboratory, U.K.
- Pacific Northwest National Laboratory (now at Texas A & M)
- MIT..(in progress)

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Basic design:
- Microscope stage for targeting
- Particle counting either by a scintillator under the cells together with a photomultiplier or an ionization chamber over the cells.

Mutagenic effects of a single and an exact number of α particles in mammalian cells

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Clearly the miners exposed to high concentrations of radon show an increased incidence of lung cancer (up to 10 times the normal rate).

At the low levels of exposure for the general population, the use of epidemiological data used for risk assessment has very large uncertainties.

EPA estimate: 21,000 lung cancer deaths in the U.S. per year directly attributable to radon exposure (confidence limits: 7,000 to 30,000).

Number of traversals of bronchial cell nuclei
- 96% in a miner > 1 particle per year
- 1 in 10^7 cells > 1 particle per year in a household environment

Objectives:
What are the effects of a single alpha particle on survival and mutation frequency?

Methods:

Irradiation is automated, ~ 2 seconds per cell, up to 10,000 cells per day.

Spatial precision is ± 4 µm.
Modeling indicates that the beam would hit the nucleus 98.4% of the time.
Energy of the alpha particles is 5.5 MeV, range ~40 µm, LET = 90 keV/µm.

All cells on a plate irradiated then removed for standard colony forming assay or mutation analysis.

Mutation assay

- Mutation assay when cells are irradiated one at a time (even 10000/day) requires a sensitive endpoint.

- A_L cells have a standard set of hamster chromosomes, plus one human chromosome

- The human chromosome codes for cell surface markers that make the cells susceptible to killing by specific antibodies and complement.

- Cells plated in dish, exposed to antibody, only those with a mutation in the human gene will not express the surface antigen and thus survive.

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Cells stained briefly with a non-toxic concentration of Hoechst dye for targeting

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- Cell survival as a function of the number of alpha particles is similar to other reports.

- D_0 is approximately 4 particles (3.7).

- 80% of cells **survive** passage of a single alpha particle.

- 10% of cells survive 8 alpha particles!!

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- Traversal of the nucleus by a single alpha particle increased the mutation frequency by a factor of 2.

- At 1 particle per cell, the microbeam results agree with broad beam alpha particle irradiations where a mean of 1 particle was irradiated.

- At 8 particles per cell, the microbeam mutation frequency is higher than that seen with a broad beam delivering a mean of 8 particles per cell. (Are cells that received lower doses "overgrowing" the population?)

Conclusions and Significance

- Nuclear traversal by a single alpha particle was only **slightly cytotoxic**, but **highly mutagenic.**

- First demonstration that a **single alpha particle** traversing a nucleus will have a high probability of causing a mutation.

- Highlights the need for radiation protection at low doses.

Targeted cytoplasmic irradiation with alpha particles induces mutations in mammalian cells

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Prevailing dogma: the nucleus is the target for genotoxic and cytotoxic effects.

Reports on the "Bystander Effect" indicate that DNA damage can be caused in cells not directly traversed.

Objective: use microbeam to selectively irradiate cytoplasm to determine if there are effects on survival or mutation frequency.

The Columbia microbeam produces alpha particles of 5.5 MeV, range ~40 μm, LET = 90 keV/μm.

Irradiation of each cell takes about 6 seconds.

All cells on the dish irradiated, then trypsinized, removed and plated for either survival or mutation analysis.

Mutation assay is the A_L hamster line with one human chromosome.

Human chromosome expresses cell surface antigens that make cells susceptible to complement inactivation. Wild-type cells are killed in the presence of antibody and complement

Cells with mutations in this gene can survive.

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- Nucleus is green, cytoplasm is red.
- Irradiate 8 μm from the end of the nucleus on each end of the cell.
- Do not irradiate if one cell's cytoplasm overlaps another cell nucleus.
- Accuracy estimate: 75% had both ends hit, 15% one end hit, 10% missed, no accidental nuclear hits.

Approach:
- Irradiate cytoplasm with 1, 2, 4, 8, or 16 alpha particles.
- Determine survival
- Determine mutation frequency
- Add scavengers of free radicals to determine if the effect is mediated by reactive oxygen species (ROS).
- Immuno-staining for specific oxidative DNA damage product 8-hydroxy-deoxyguanosine (8-OHdG).

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Lethality is minimal compared to nuclear traversals:
- 4 particles in cytoplasm, > 90% survival; (35% if 4 particles are in the nucleus)
- 32 particles in cytoplasm, >70% survival; (<< 1% if 32 particles are in the nucleus)

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Mutation frequency:
- Background level is 43 ± 15 mutants/10^5 cells.
- The effect saturates after 4 particles per site.

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Mutation spectra:

Most of the mutants from 8 particles through the cytoplasm were similar to the spontaneous mutants: small alterations only in the CD59 gene.

80% of the mutations seen after 8 particles through the nucleus were multi-locus.

Different mutagenic mechanisms involved?

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Scavenger experiments:
- Add DMSO to reduce ROS directly: DMSO can protect against indirect damage
- Add BSO to deplete intracellular glutathione (GSH). GSH is the cells natural scavenger of ROS.

DMSO added before and after irradiation reduced level of mutants (4-5 fold).

Pretreatment with BSO for 18 hrs reduces GSH levels to 5% of controls.

Mutation frequency increased 4-5 fold.

DMSO and BSO alone were non-toxic and non-mutagenic.

DMSO treatment had no effect on the mutation frequency following 4 particles through the nucleus.

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Detection of oxidized nucleoside (8-OHdG).

- 8 particles through the cytoplasm
- Fluorescence intensity increased by ~ 2-fold
- Time course indicated maximum signal intensity seen at 5 minutes after irradiation.
- Signal intensity decreased to control levels by 45 minutes after irradiation.

Conclusions:
- Nuclear traversals -- direct effect
- Cytoplasm traversals -- indirect effect mediated by ROS
- Mutation spectra of cytoplasm traversals look like spontaneous mutations.
- Cytoplasmic mutations mediated by ROS? Scavenger experiments support this. Detection of 8-OHdG supports this.
- Mechanism? Hydroxyl radicals involved?
- Migration length of OH· is too short, ~ 4 nm, targeted sites were ~8 µm from the nucleus.
- Cascade to longer-lived free radicals?
- Mitochondria involved? Could explain saturation effect.

Significance:

- Nuclear traversals produced 3-4 fold more mutations at equal numbers of particles.

- *However, cytoplasmic traversal may be more important for carcinogenesis*

- At **equitoxic** doses (e.g., 90% survival) cytoplasmic irradiation induced 7-fold more mutations than nuclear traversals.

- Cytoplasmic irradiation could actually be more of a health risk.

The oncogenic transforming potential of the passage of single α particles through mammalian cell nuclei

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Estimation of risk from exposure to radon is based on epidemiological data in miners, and extrapolated to the low exposure levels present in homes.

Bronchial epithelial cells in miners may have been traversed by several particles in a short period.

For the individual in a domestic radon situation, it is highly unlikely that any cell will be traversed by more than one alpha particle in an entire lifetime.

Objectives:

- Investigate the oncogenic effects of exactly one alpha particle.

- Compare the effects of exactly one to a Poisson mean of one alpha particle.

- This is a check on whether the cells receiving more than one particle dominate the response.

- "Control" study: comparison of exactly four with a Poisson mean of four particles.

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The microbeam facility at Columbia.

Continuous improvements in automation make it possible to irradiate ~ 3000 cells per hour.

"Broad beam" studies produce the statistical Poisson distribution of particle traversals.

For a mean of 1 particle:
- 37% will see 0 traversals
- 37% will see 1 traversals
- 26% will see 2 or more traversals

The average dose will be the same as the group that all received **exactly one** traversal.

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Cells are stained with a Hoechst dye to visualize the nucleus for targeting. Broad beam groups are also stained as a control.

Transformation was judged by microscopic inspection of colonies after 7 weeks of growth. Methods previously reported. Such transformed cells produce tumors when injected into animals.

Transformed cells lose contact inhibition.

[Image removed due to copyright concerns]

[Hall, 2000]

Transformed cells lose anchorage dependence.

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[Hall, 2000]

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Transformation frequency/10^4 surviving cells.
- Control rates are similar.
- Broad beam results are greater than microbeam results only at 1 particle.
- At 2, 4, and 8 particles the microbeam results are the same (within the error bars) as the broadbeam results.

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- Cells traversed by exactly one alpha particle show a lower risk than those receiving a mean of one.

- Exactly one alpha particle is not significantly different than controls receiving zero!!

- This implies that the majority of transformed cells resulting from a mean of one traversal must come from the subpopulation that received more than one traversal.

- If traversal by only one particle does not significantly raise the risk of oncogenic transformation, estimations by extrapolation from higher doses received by miners will overestimate the risk.

Induction of a bystander mutagenic effect of alpha particles in mammalian cells

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Previous work by this group with this cell line
- Exact numbers of alpha particles: survival and mutation endpoints.
- Cytoplasmic irradiation.

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- Now carried out to 20 particles per nucleus.

- D_0 is approximately 3.6 alpha particle traversals.

- Survival after 20 particles through the nucleus is ~ 1%.

A_L cells: hamster with one human chromosome that expresses cell surface markers for complement inactivation.

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- ~500 cells plated in irradiation dish
- At random, 5, 10 or 20% of the cells in the dish irradiated with 20 alpha particles each
- >99% of the surviving cells are unirradiated, but the mutation rate is 3 times higher than control.
- Bystander effect in cells not traversed by alpha particles.
- The effect appears to "saturate".

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- For the population where 20% of the cells were exposed to 20 alpha particles, 99.8% of the progeny are calculated to be from unirradiated cells.

- The mutational spectra are clearly different from those that occur spontaneously.

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- The bystander mutagenicity was not affected by addition of DMSO.
- The effect is not due to a free radical ROS.

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- Lindane blocks gap junctions and reduces the bystander effect.
- Cell-cell contact plays an important role in this effect.