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Track Theory Predictions for Single-Hit Cell Survival

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EXTENDED ABSTRACTS

Proceedings of the 4th International Workshop: Microbeam Probes of Cellular Radiation Response[1]

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The extended abstracts that follow provide a summary of the Proceedings of the 4th International Workshop: Microbeam Probes of Cellular Radiation Response, held in Killiney Bay, Dublin, on July 17–18, 1999, which was jointly organized by the Columbia University Radiological Research Accelerator Facility and the MIT Laboratory for Accelerator Beam Applications.

There is increasing interest in the use of microbeam systems, which can deliver beams of different radiations with a spatial resolution of a few micrometers or less, for radiobiological research. Single-particle microbeams can be used to address such questions as the relative sensitivities of different parts of the cell (e.g. nucleus compared to cytoplasm), and the effects of irradiation of neighboring (bystander) cells. For particle (e.g. α-particle) beams, irradiation with exactly one (or more) particle per cell can be achieved, allowing questions of risks of very low doses of ionizing radiations, such as radon, to be addressed. Several microbeams are now in operation, and others are being developed. The workshop provided a forum to assess the current state of microbeam technology and current biological applications, and to discuss future directions, both technological and biological.

Roughly 75 scientists (about equal numbers of physicists and biologists) attended the workshop, the fourth in a biannual series (*1*). A list of attendees can be obtained from David Brenner (djb3@columbia.edu). A fifth meeting is planned for the year 2001.

Reference

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The Performance of the Gray Laboratory Charged-Particle Microbeam

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The microbeam offers a unique method for selecting and irradiating individual cells with a controlled number of charged particles. For a microbeam to truly fulfill its role in modern radiobiology, it must excel in a number of technical aspects. The versatility of a modern microbeam can be assessed by considering the three most important aspects of its performance: the targeting accuracy, the particle counting efficiency, and the rate at which cellular targets can be identified and irradiated.

The targeting accuracy of any microbeam is fundamentally limited by the chosen method of "aiming". While focusing potentially offers the finest probes, the overall accuracy of a focused microbeam may be no better than a collimated system, once other requirements are fulfilled. The Gray Laboratory has endeavored to develop a collimated microbeam with state-of-the-art targeting accuracy for an *in vitro* system, and without undue compromise in other areas. The development of the microbeam and preliminary measurements of the collimator performance using protons have been reported elsewhere (*1, 2*). Briefly, our system uses collimated particles accelerated by a 4 MV Van de Graaff accelerator to irradiate cells attached to a thin film that forms the base of a cell dish, itself supported on a motorized stage. A computer-controlled charge-coupled device (CCD) camera and epifluorescence microscope are used to automatically find and record the position of stained cells. To irradiate the cells, the microscope objective is replaced with a photomultiplier tube that detects photons produced when a particle traverses a thin scintillator situated between the collimator and the cells. Cells are positioned and irradiated automatically at a rate of just over 1 s per cell. Recent experiments have used mainly ^{3}He ions to selectively target either the cell nucleus (*3*) or the cytoplasm. Alongside these experiments have been developments to improve the targeting accuracy and subsequent performance-related studies using CR-39 track-etch detectors.

The overall targeting accuracy of any microbeam is limited not just

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Evidence that increased radioresistance is dependent on linear energy transfer. *Radiat. Res.* **138** (Suppl.), S81–S84 (1994).

Microbeam Irradiation Patterns to Simulate Dose

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Underlying the argument for the use of a linear extrapolation to estimate the risk resulting from low doses of radiation is the assumption that the effect is the result of an individual cell responding to damage done by energy deposited in that cell. Single-particle microbeam irradiation has shown that at least some biological end points can be produced by passage of a single α particle through a cell. There is adequate evidence that the health effect of greatest concern, cancer, is the result of the abnormal growth of a single cell. It is also known that, under normal circumstances, cells are influenced in many ways by their local environment. Cells are stimulated to divide, differentiate or self-destruct by signals from neighboring cells and the extracellular matrix. There is also evidence that some members of an irradiated population alter their levels of repair-related proteins and change their DNA metabolism even though they cannot have been hit directly by an ionizing particle. This strongly suggests that cells do not act autonomously, and it may be that interactions between hit and unhit cells influence the probability of an adverse effect in a way that depends on the number of hit cells in the population, that is in a dose-dependent way. Several characteristics of the radiation and the target system are likely to influence the magnitude and frequency of modifications to the response of individual cells. The obvious properties of the radiation are the stopping power, mass and velocity of the charged particle, the spatial distribution of the charged-particle tracks, and the temporal distribution of events. For relatively high-LET radiations (protons and α particles), the spatial distribution of events at a "low" dose is relatively sparse, typically an average of many cell diameters between charged-particle tracks. If the dose rate is low, adjacent tracks may be separated in time by many cell divisions. The relevant characteristics of the target system include everything that influences the amount of energy deposited, conditions (such as oxygen concentration) that influence the consequences of energy deposition, and factors (such as cell contact and extracellular matrix structure) which influence the communication of information and materials between cells.

Microbeam irradiation provides the means to vary the spatial and temporal distribution of the irradiation in a controlled fashion. There are many different ways to deliver the same average number of charged particles per cell. The geometry conventionally used for track-segment irradiation of cells in culture, charged-particle tracks perpendicular to the plane of a thin substrate supporting a monolayer of (flattened) cells, is not a realistic simulation of the energy deposition around cells irradiated in a tissue. In the tissue, typically several adjacent cells are traversed by the charged particle, potentially resulting in communication of damage-related signals between them and possibly in strengthening the signal transmitted to unirradiated adjacent cells. To select among the alternative irradiation patterns, we need some plan for investigating the mechanisms that may be important for health effects. The best approach is to start with models of some proposed mechanisms and devise experiments that can prove one or more of the alternatives to be wrong.

It is very early in the development of understanding of the effects of neighboring cells on radiation response, but a few models can be proposed on purely logical grounds. Communication may be through direct contact between cells or through release of compounds into the medium. To simplify, we will consider direct cell-to-cell communication only. The signal strength, that is the probability of a signal producing a specific observable change in an unirradiated cell, can be assumed to be proportional to the amount of energy deposited in the irradiated neighboring cell. An alternative model is that the signal strength produced by an irradiated cell is constant for any energy deposition above a threshold level, but the strength of the signal at an unirradiated cell is proportional to the number of cells surrounding it which have received more than the threshold level.

Both of these models could result in nonlinear effects at low doses, but the dependence on dose and LET they would produce would probably be quite different. Another factor that is important in the response is the distance (*d*) over which the signal acts. The simplest model is that the signal decreases as $1/d^2$ as it propagates through adjacent cells. However, the possible extremes are that it can be transferred to neighboring cells only through direct contact with the irradiated cell, or that it is amplified by each cell receiving it and is passed on without attenuation until it reaches a boundary it cannot pass (for example, edge of cell clone, change of cell type in a tissue).

These models suggest a number of experiments, involving different energy deposition patterns, which can be used to distinguish between the alternatives. These experiments would determine if cell communication is significant in determining the dose–response relationship at low doses. First, it will be necessary to determine how the probability of an effect of an energy deposition in a target cell depends on the spatial distribution of the initiating energy. If a confluent monolayer of cells, *t* micrometers thick, is used as a two-dimensional model of cells in a tissue, a given dose can be delivered by *n* particles, with range *r*, per cm^2 distributed as one per site with the sites uniformly spaced $n^{-1/2}$ cm apart, as *p* tracks per location with the spacing between locations increased to $(n/p)^{-1/2}$, or by *r*/*t* tracks spaced *t* apart with the pattern repeated at a spacing of $(nt/r)^{-1/2}$. This last distribution simulates the distribution of energy that would occur in a three-dimensional tissue. If the probability of producing a specific bystander effect is proportional to the total amount of energy deposited, the three patterns should produce the same frequency of effects. If there is a threshold value of energy deposition in a cell that is sufficient to trigger communication to neighboring cells, the number of affected cells should decrease with increasing *p* because the total number of cells exceeding the threshold energy deposition decreases. If the probability of effect is related to the number of cells exceeding the threshold level, the result will depend on the range of the interaction. We expect that use of models of the effects of cell communication will allow us to design more efficient experiments to test the linear, no-threshold model.

Track Theory Predictions for Single-Hit Cell Survival

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The track theory of cell survival after heavy-ion irradiation is based on the probability of survival after a single-particle transit, called "ion-kill" (not track core), joined to the cumulative effect of δ rays from adjacent ions in a beam, called "gamma-kill" (not penumbra). The model offers a set of equations containing four parameters, E_0 and *m* for the single-hit multitarget statistical model for gamma-kill, and additionally σ_0 and κ for ion-kill. A single set of parameters is used to fit a family of survival curves obtained with ions of different LET simultaneously. With these parameters, the model then predicts cell survival for arbitrary radiation fields (as in the spread-out Bragg peak, neutrons, even admixed with γ rays) whose particle-energy spectrum is known. It has predicted "Katz tails" (also called "Darmstadt hooks"). Parameters have been fitted to upward of 40 sets of data for cell survival and transformation (*1*). These parameters and the equations of the model are now used to predict single-hit survival. We require as input data the atomic number *Z* of the bombarding ion and its relative speed β, and the identity of the cell so as to select the appropriate parameters κ and *m*. We then calculate the probability for ion-kill from Eq. (4) of ref. (*1*) as $P = [1 - \exp(-Z^{*2}/\kappa\beta^2)]^m$, with the effective charge Z^* from Eq. (2) of the reference. Note that ion-kill is responsible for all high-LET effects: increased RBE, decreased OER, loss of repair with the consequent fibrosis in heavy-ion and

neutron therapy, presumably from repopulation (analogous to scar formation).

Some results:

1. *Experiment*: At the Gray Laboratory: Single 3 MeV protons onto Chinese hamster V79 cells. No killing observed. Number of trials unstated (private communication). *Theory*: Probability for cell killing ~0.001. *Related data*: Warters *et al.* found that some 500 tritium β-particle decays in the nucleus of a CHO cell are required for observable cell killing (*2*).

2. *Experiment*: At Columbia (*3*): Single α particles (110 keV/μm) onto unidentified cells; 60–85% survival. *Theory*: For Chinese hamster cells, κ = 1400, *m* = 3, probable surviving fraction 33%. For T-1 kidney cells, κ = 1900, *m* = 2.5, probable surviving fraction 42%. Our calculations are made for α particles at 116 keV/μm, $Z^{*2}/\beta^2 = 2{,}290$.

3. *Experiment*: At Columbia University:[2] Single α particles (110 keV/μm) onto C3H 10T½ cells. Observed probability of oncogenic transformation 0.0001. *Theory*: At 116 keV/μm, probability for transformation 0.00021. Here κ = 750, *m* = 2. There is a geometric factor here, for α particles are directed through the nucleus rather than through the genome. Calculating for the nucleus, 91% of the cells are mutated. But if we take the geometric factor to be the ratio of s_0 for cell killing and for transformation, we find it to be 2.3×10^{-4}. Thus we expect that the fraction of cells undergoing an oncogenic transformation will be 2.1×10^{-4}.

4. *Experiment*: At Naples:[3] Single 4.3 MeV α particles onto Chinese hamster V79 cells. Probability of surviving 1 nuclear traversal 67 ± 10%. *Theory*: Probability for surviving 58%. Calculated for 1.167 MeV/nucleon for which $Z^{*2}/\beta^2 = 1{,}538$. Values of κ and *m* are as quoted above for these cells.

Some additional comments: We note that since single-particle transits are deterministic, while dose is a statistical concept, it is inappropriate to refer the effect produced by a single-particle transit to the "dose" it deposits, just as if one referred the kinetic energy of a single electron to its "temperature" (*4*). So also cross section (*5*) is a statistical concept. Thus it is inappropriate to apply the term cross section to the fraction of successes in targeted trials, as in microbeam experiments.

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Evaluation of the Risks Associated with Single-Particle Effects Using a Charged-Particle Microbeam

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We are developing *in vitro* models relevant to radiation risk using a microbeam capable of delivering individual charged particles to individual cells *in situ*. The system will allow us to critically determine the response of human cells to the single-particle traversals typically encountered in environmental exposures such as with radon, avoiding the confounding effect of the Poisson distribution of particle traversals inherent in conventional exposure systems. After environmental radon exposure, for example, virtually no cell receives more than one charged-particle traversal in its lifetime. Thus use of the microbeam will aim to produce data for direct input into the analysis of human health risks of environmental and occupational exposures involving charged particles.

The Gray Laboratory charged-particle microbeam delivers micro-collimated (1–5 μm) radiation using a 4 MV Van de Graaff accelerator (*1*). A computer-controlled microscope system is used to record the coordinates of and to irradiate individual cells. The cells can be stained with different fluorochromes to deliver individual particles to specified cellular locations, for example, Hoechst 33258 (nucleus) or rhodamine 123 (cytoplasm). Currently about 90–95% of the particles are delivered with a positional accuracy of ±2 μm and a detection efficiency of >99% (*2*).

The versatility of the microbeam allows for the study of acute irradiation damage, delayed instability and the bystander effect after single-cell irradiation. Previously, we have determined the induction of micronuclei in Chinese hamster V79 cells exposed to individual protons (*3*). We are currently studying radiation effects in a normal human fibroblast cell strain (AG-1522) by assessing the yields of both lethal and nonlethal chromosome damage (using FISH technology) and the yields of acentric chromosome fragments as micronuclei. Cells were irradiated by microbeam $^3\text{He}^{2+}$ particle traversals (used as surrogates for α particles, with an energy 3.5 MeV and an LET of 95 keV/μm) and by conventional "broadfield" α particles (^{238}Pu, 3.9 MeV, 110 keV/μm) for comparison.

We have measured chromosome aberrations produced by average numbers of α particles in chromosome 1 using FISH. Chromosome exchanges were scored by the Savage and Simpson nomenclature system (*4*). By this method, we found that around 20–30% of the total exchanges produced after 0.5–0.75 Gy α particles are complex, containing three or more breaks in two or more chromosomes. These results compared well with a previous study of chromosome 1 aberrations in human fibroblasts after α-particle irradiation (*5*). Multicolor painting of chromosomes 1, 4 and 8 showed 20–40% of complex exchanges at these radiation doses. This multicolor FISH approach is currently being developed to determine damage to chromosomes 1, 4 and 8 after microbeam $^3\text{He}^{2+}$-particle irradiation. Metaphase spreads will be prepared from irradiated cells and multicolor painted *in situ* on the microbeam dish, potentially allowing us to revisit the targeted cells to assess the quality of damage resulting from a single $^3\text{He}^{2+}$-particle traversal through the cell nucleus.

We are studying acute effects and the production of chromosomal instability at these low doses of α particles using the daughter cell micronucleus assay. Micronuclei were scored 72 h after conventional or microbeam irradiation in acridine orange-stained daughter cells. An individual $^3\text{He}^{2+}$-particle traversal induced micronuclei in 8% of the cells it traversed. At higher numbers of particles, the yield reaches a peak and then decreases, due to cell cycle delay. This effect was also seen after broadfield α-particle irradiation (*6*). Comparison between the two irradiation systems showed that an exact number of α particles (delivered by the microbeam) induces more cell damage as micronuclei than an average number of particles (delivered by conventional irradiation). At delayed times, up to 30 days later, the yield of micronuclei remains elevated due to the *de novo* production of instability. This instability is more pronounced for high-LET α particles in comparison to X rays.