Heritable Non-lethal Damage to Cultured Human Cells Irradiated with Heavy Ions

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During interplanetary flights the nuclei of all of a crew member's cells could be traversed by at least one high-LET (Linear Energy Transfer) cosmic-ray particle. In mammalian cells irradiated in vitro about 1 in 10,000 of the surviving cells traversed by heavy particles is transformed to malignancy or mutated. What, if anything, happens to the remaining >99% of surviving cells? A retrospective analysis of archived data and samples from heavy-ion irradiation experiments with cultured human cells in vitro indicated that heavy ions caused a dose- and LET-dependent reduction in growth rates of progeny of irradiated cells, based on colony-size distributions. The maximum action cross section for this effect is between 100 and 300 \( \mu m^2 \), at least as large as the cell nuclear area and up to 3 times the cross section for cell killing. Thus, heritable slow growth is the most prevalent effect of high-LET radiations on cultured animal cells, which may have implications for crew health during deep space travel.

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INTRODUCTION

Calculations indicate that on a round trip to Mars, the nuclei of all of a crew member's cells will be traversed by at least one energetic cosmic-ray particle unless extraordinary shielding measures are implemented. The irradiation of cells in vitro with heavy ions has for several decades served as a model for studying the potential effects of cosmic-ray particles on cells. It has been amply demonstrated that surviving cells traversed by heavy particles are transformed to malignancy or mutated; however, these well-known outcomes are only observed in less than 1 in 10,000 surviving cells. The question remains, what, if anything, happens to the remaining 99+% of surviving cells?

A retrospective analysis of archived data and samples from heavy-ion irradiation experiments with cultured human cells was undertaken to seek evidence for reduced growth of surviving clonogenic cells. We evaluated colony-size distributions of human T1 cells that were exposed to various doses of both low- and high-LET radiation to 1) seek evidence for non-lethal heritable damage (small colony formation and reduced growth rates), 2) show that the induction of small colonies is dose- and LET-dependent, and 3) estimate the action cross section for this damage. Division delay (a transient effect on surviving colonies) and reduced multiplication rate (a heritable trait) were analyzed separately as a function of dose and LET using a colony-size percentile method.

MATERIALS AND METHODS

Cultured cell irradiations

Data were taken from archived data and archived samples from experiments in which cultured human cells had been exposed to x-ray, heavy-ion or neutron irradiation and allowed to grow into colonies for 12–14 days at which time they were stained and preserved. All experiments were performed using the same cloned, cultured human cell line,
designated T-17–9). Culture procedures were as previously described, and colony-formation experiments were performed using 35 mm polystyrene petri dishes or T-25 flasks for neutron irradiations. These dishes or flasks containing stained colonies were archived and maintained under dry, temperate conditions from 1966 to 1982. Cells were exposed to the respective radiations, photons, heavy ions, neutrons (30 MeV deuterons on Be, National Institute of Radiological Sciences, Chiba, Japan) as described in earlier publications.

Colony size distribution analysis

Early colony sizes were determined at the time of each experiment by enumerating, by microscopy, the cells in each of about 100 colonies during the first 6 days of post-irradiation growth and calculating the mean number of cells per colony to obtain growth curve parameters (lag and slope). Colony size distributions were determined from archived raw data, and growth curves for each decile of colony size were plotted independently. Final colony size distributions were generated by computerized image analysis of archived petri dish samples containing human T1 cell colonies from low (x- and γ-rays, 3D), intermediate (neutrons, 4He, 7Li), and high-LET (40Ar, 20Ne, 16O, 12C, 11B) irradiation survival experiments. After digital conversion each image was edited to insure that each object was a colony of cells, then the number of pixels per colony was tabulated for each colony and defined as colony size. Typically 50–100 colonies were measured and recorded for each dosage point. Absolute size distributions were converted to percentile distributions.

RESULTS

Early colony growth and division delay

Plots of integrated clone-size distribution curves were generated from counts of cells per colony at different times after plating (Fig. 1). By assuming that the growth rates of the individual clones, as depicted by their sizes at given times, did not vary during the period of observation (demonstrated to be a correct assumption — data not shown), independent growth curves for each decile of colony size were constructed. These curves were used to determine growth rates (doubling times) and division delays of clones of different sizes. In evaluating the growth characteristics of the colonies, we found a spectrum of growth rates and division delays in both large and small colonies. However, compared to the controls (D=0), colonies arising from irradiated cells had reduced growth rates (increased doubling time – Fig. 1a) and increased division delays (Fig. 1b). These two figures also show that the smaller colonies had the longer doubling time (lower growth rate) and longer division delay. It is noted in passing that the 90th and 100th percentile colonies reflect the fact that these colonies initially had more than one cell.
vival curves based on final colony size. Thus, the measurement of surviving fraction of “large” colonies is uncoupled from the ultimate effects of division delay, which affects the sizes of both small and large colonies.

**Final colony size distributions**

Fig. 2 is a family of raw colony size distributions vs. radiation dose (γ rays in this case). As predicted from growth data, the size of the largest colonies decreases with dose; this decrease is attributed to division delay. The obvious rise in the fraction of small colonies with increasing dose is separately attributed to heritable slow growth, consistent with the known growth characteristics of cells cloned from small colonies produced by ionizing radiation. We categorized colonies as large or small in percentile clone-size distributions based on their dose response. All colony size area classes that decreased with dose were classified as “large” colonies. Those area classes that increased with dose were classified as “small” colonies (Fig. 3). The results showed that clone-size distributions of human T1 cells exposed to all radiations widens and shifts toward small colonies as the dose increases (Fig. 2). Associated with this shift is a dose-
dependent decrease in the fraction of large colonies (Fig. 4), which is considered to be a survival curve for normal-sized colonies, a survival curve that varies with LET. High-LET radiations, such as $^{12}$C and $^{16}$O ions, are more efficient in the production of small colonies, per unit dose, than x-rays or neutrons. The action cross section for the loss of large colonies was derived from the 40% and 80% survival levels on several plots like those in Fig. 4, and this increased with LET, with a tendency toward a maximum between 100 and 300 $\mu m^2$ (Fig. 5), depending on the level of survival chosen\textsuperscript{15}.

### DISCUSSION

Cultured human T1 cells exposed to low-, intermediate-, and high-LET radiation, such as heavy ions, show evidence of heritable non-lethal damage, which is manifested by a dose dependent increase in the percentage of small colonies. This increase is LET dependent and can be attributed to reduced colony growth rates. The very high incidence of small colony trait, which is retained over many subcultivations in cloned progeny of irradiated cells\textsuperscript{13,14}, inspires questions concerning its genetic origins. The accompanying defects (low plating efficiency, increased radiosensitivity, high reversion frequency) noted decades ago tended to “rule out point mutations unless any one of a thousand or more genes can be responsible for this phenotype”\textsuperscript{14}. Today, the term “genomic instability” is applied to a number of similar delayed events observable in the progeny of irradiated cells\textsuperscript{16,17} and is LET-dependent\textsuperscript{18}.

Thus, while we continue to learn more about modes of cell death following heavy-ion irradiation\textsuperscript{19}, it is also likely that cell impairment in organized tissues\textsuperscript{20} is due to a combination of cell death and heritable damage to surviving cells. If 5 particles of at least 200 keV/$\mu m$ (2,000 MeV-cm$^2$/g), on average, traverse the nuclei of a population of cells, 37% will survive\textsuperscript{6}, nearly 100% of the surviving cells will undergo reversible division delay, and some 98% will suffer heritable damage that affects their growth rate. The high action cross section for this effect (100–300 $\mu m^2$) is also consistent with the claim\textsuperscript{21} of genetic effects due to cytoplasmic alpha-irradiation. This cross section is at least as large as the cell nuclear area (80–120 $\mu m^2$)\textsuperscript{6} and at least 3 times the cross section for cell killing\textsuperscript{6,10}, making heritable slow growth the most prevalent effect of high-LET radiation.
on cultured animal cells. In planning long-term manned space flights, high priority should be placed on understanding the risks of heritable damage to the cells of astronauts exposed to cosmic rays.

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