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TARGET CONSIDERATIONST. Straume
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ABSTRACT

Highly radiosensitive primordial mouse oocytes, the principal cells at genetic risk in the female, have been studied using 0.43-MeV neutrons. Analysis of the survival curve ($D_{37} = 0.055$ Gy) indicates that the diameter of the radiosensitive target (assumed spherical and of unit density) is larger than that of the nucleus but not of the oocyte, implicating a non-nuclear but sub-cellular target. This is consistent with results from ^3H -thymidine incorporated in DNA. Our efforts to identify the extraordinarily radiosensitive lethality target in these primordial oocytes suggest it is the plasma membrane. Monte Carlo calculations for 0.43-MeV neutrons show that at the D_{37} only a single proton recoil will traverse the plasma membrane, consistent with the observed exponential survival curve. A highly sensitive non-DNA target for mouse oocyte killing may importantly influence interpretations of genetic mutation data from mice and their use in estimating genetic risk in humans.

INTRODUCTION

Resting mouse oocytes are extremely vulnerable to cell killing.⁽¹⁾ They are 50-100 times more radiosensitive than most other mammalian cells. Since such extraordinary radiosensitivity may result from an unusual target, we have examined target size, geometry, and location, using 0.43-MeV neutrons (and other radiations) and Monte Carlo computer calculations.

The animals used were noninbred Swiss-Webster mice. Methods for radiation exposure, preparation of ovaries for microscopic examination, and oocyte enumeration have been described elsewhere.^(1,2,3) Exposure of mice to 0.43-MeV neutrons were done at the Radiation Research Accelerator Facility (RARAF). Monte Carlo calculations were performed using a CDC 7600 computer at Lawrence Livermore National Laboratory.

TARGET CONSIDERATIONS

The target size can be estimated from survival curves and microdosimetric parameter values, i.e., from the D_{37} of an exponential survival curve and the event frequency measured in a tissue-equivalent detector of known volume and gas pressure.⁽⁴⁾ Event frequencies for a variety of spherical volumes simulating volumes of cells and organelles have been reported in the literature.⁽⁵⁾

For 0.43-MeV neutrons, single hit killing kinetics are suggested by the exponential oocyte survival curve. The event frequency in the oocyte lethality target is estimated by taking the reciprocal of the D_{37} (i.e., $1/.055 \text{ Gy} = 18 \text{ Gy}^{-1}$). The diameter of the target (assumed to be a solid tissue sphere) is thus about 9 μm , larger than the resting oocyte nucleus ($\sim 7 \mu\text{m}$) but smaller than the entire oocyte ($\sim 12 \mu\text{m}$). This result is consistent with oocyte survival data for ^3HOH and ^3H -thymidine. For

example, as shown in Table 1, when tritium is confined in the nucleus (^3H -thymidine) the dose required for 37% survival is about 100 times greater than when tritium is evenly distributed in body water (^3HOH). This clearly establishes that the nucleus is not the radiosensitive target for mouse oocyte killing.

In identifying the target, various cellular organelles were considered. The plasma membrane is the only sub-cellular structure that appears consistent with all of our dose-response and cell morphology data. The plasma membrane geometry (a thin spherical shell) prevents direct target size estimation from data obtained with proportional counters, which simulate only solid tissue spheres. We therefore used Monte Carlo computer calculations⁽⁶⁾ to determine the relationship between the event frequency for a solid tissue sphere (oocyte) and a thin tissue shell (plasma membrane). Results are seen in Figure 1 and indicate that for a 12 μm diameter oocyte, the plasma membrane is traversed 0.55 times per cell hit. To obtain the event frequency for the plasma membrane, we simply multiply the event frequency from proportional counter measurements by 0.55. Since the event frequency from proportional counter measurements⁽⁴⁾ for 0.43 MeV neutrons in a 12 μm solid tissue sphere is about 35 Gy^{-1} , the plasma membrane will be traversed about $35 \times 0.55 = 19$ times per Gy. This is essentially identical to the value of 18 Gy^{-1} obtained from the survival curve.

More recently, using heavy ions of Si accelerated in the Bevalac at Lawrence Berkeley Laboratory, we have preliminary oocyte survival data suggesting a target size equal to the cross sectional area of the entire oocyte. Although absolute proof is not yet available, these results, and data discussed previously,⁽³⁾ strongly suggest the plasma membrane as the radiosensitive target for mouse oocyte killing.

Because primordial oocytes are of prime importance genetically, the observation that in mice there is a highly radiosensitive target for their destruction, which is different from that for genetic damage, may have important implications for interpreting experimental results and estimating genetic risk in the human female. Also, because of their high radiosensitivity, these cells may be useful for testing biophysical models at very low doses in the intact animal.

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TITLES AND CAPTIONS

Table 1. Mean dose to oocyte nucleus and plasma membrane at the 37% survival level.

Figure 1. Relationship between recoil proton traversals of the plasma membrane and hits in the total cell for 0.43-MeV neutrons (Monte Carlo computer calculations⁽⁶⁾).

Table 1. Mean dose to oocyte nucleus and plasma membrane at the 37% survival level

<u>Radiation source</u>	<u>Dose (Gy)</u>	
	<u>Nucleus</u>	<u>Plasma membrane</u>
^3HOH	.065	.065
^3H -thymidine	$\sim 6.^a$	$\sim .06^b$

^a Dose from autoradiography.

^b Dose calculated using Monte Carlo computer code⁽⁷⁾.

Figure 1. Relationship between recoil proton traversals of the plasma membrane and hits in the total cell for 0.43-MeV neutrons (Monte Carlo computer calculations⁽⁶⁾).