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T. Straume
R. Khan
O.G. Raabe
K.J. Walsh
L.M. Wiley

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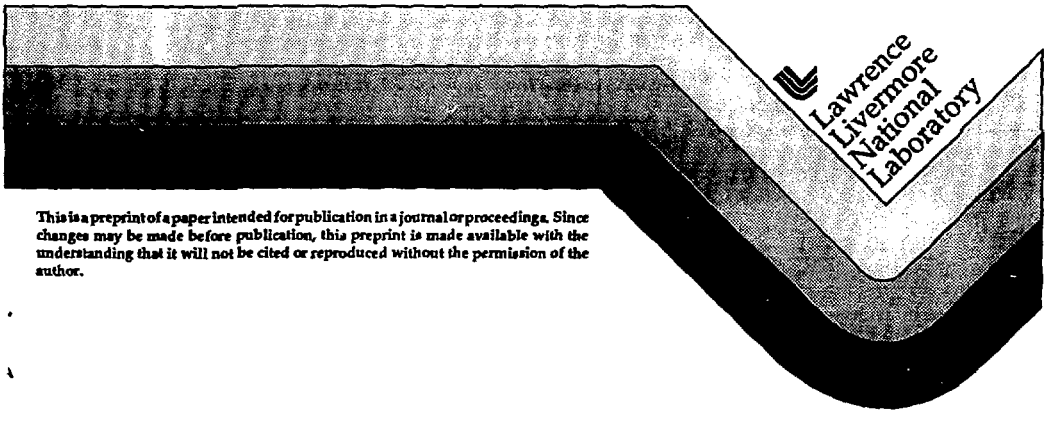

Inherited effects from mouse immature oocytes following low-dose irradiation

T. Straume,[a] R. Khan,[b] O.G. Raabe,[b] K.J. Walsh[b] and L.M. Wiley[b]

[a]University of California, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA

[b]University of California, Institute of Toxicology and Environmental Health, Davis, CA 95616-8615, USA

INTRODUCTION

Immature oocytes represent the genetic pool in female mice as well as in women and therefore are principal cells of concern for genetic studies [1]. Previous studies have demonstrated that genetic effects in female mice can be masked by the hypersensitive plasma membrane lethality target of immature oocytes [2-3]. Studies have also shown that genetic effects can be detected when the plasma mambrane is sufficiently spared [4]. Here, new data obtained using the mouse preimplantation embryo chimera assay are presented and discussed in light of previous findings for irradiated mouse oocytes.

MATERIALS AND METHODS

The chimera assay employs differentially-stained mouse aggregate chimeras composed of two 4- or 8-cell embryos [5]. One of the embryos is obtained from a mating involving the irradiated female and an untreated male and the other is from untreated parents. The one from untreated parents is labeled with FITC and serves as the unexposed control. The endpoint is proliferative disadvantage of the cells from the treated (unlabeled) embryo measured after 2 to 3 cell divisions (28 to 32 h) and is quantified by the proliferation ratio, unlabeled cells / total cells. If exposure of the parent prior to conception reduces the cell-proliferation capacity of the early embryo, then the ratio would be less than 0.5. Previous studies on male mice using this assay have demonstrated effects at very low doses, in the 0.01-Gy range [6].

Randomly bred ICR (CD-1) mice from Charles River Laboratories, Portage, MI, were used for all these experiments. Males were housed 1 to a cage and females 4 to a cage. Sufficient numbers of embryos were obtained by superovulation and subsequent mating of females, as previously described [5,6]. The protocol for animal use and care was according to approved procedures at UC Davis.

Six-week-old female mice were irradiated using ^{137}Cs gamma rays. Doses were 0 Gy (unexposed controls), 0.05 Gy, and 0.15 Gy. At 1, 2, 3, 4, 5, 6, 7, 8, and 12 weeks postexposure, the mice were mated, chimeras made, and proliferation ratios determined as described above. Also, in a related study, embryos were exposed *in vitro* to ^{137}Cs gamma rays, ^{3}H-Tdr, or ^{3}HOH.

Exposures to ^{3}H-Tdr and ^{3}HOH were accomplished by incubating the embryos for 2 h in T-6 medium containing selected activity levels. Doses for ^{3}HOH were based on uniform concentration of tritium in cellular water. Preliminary dosimetry for ^{3}H-Tdr was based on data of Kelly and Rossant [7], which show that 2 h labeling of 8-cell embryos with 0.25 μCi/ml of ^{3}H-Tdr resulted in 30 grains during 14 days of autoradiographic exposure. Final dosimetry will be obtained from autoradiographic and scintillation-counting measurements now being performed in our laboratory.

RESULTS AND DISCUSSION

Three independent experiments have now been carried out, all showing a significant cell proliferative disadvantage of the embryos obtained from the females treated 7 weeks previously, i.e., embryos from immature oocytes. The results are illustrated in Table 1. No effect was detected prior to 7 weeks when embryos were obtained from maturing oocytes. Also, the effect appears to disappear by 12 weeks postexposure.

These findings have rather intriguing implications. The chimera assay appears to be much more sensitive than either conventional dominant lethal tests or the seven-specific-locus test. For example, a statistically significant effect was observed here following only 0.15 Gy of gamma rays whereas acute x-ray doses in excess of 0.5 to 1 Gy were required for dominant lethals [8] and for seven-specific-locus mutations [1]. Also, substantially fewer animals (10 to 15 per dose group) are required in the chimera assay.

The significant cell proliferative disadvantage observed during the seventh week, but not before or after, suggests a high sensitivity during the stage in oocyte development immediately prior to the transition between the immature resting phase and the phase when oocytes begin to grow and develop into mature follicles. This sensitive "window" has not been observed in the dominant lethal test [4,8], in studies involving measurements of cytogenetic damage during meiotic metaphase [4], or in seven-specific-locus tests [1], and thus far appears to be unique to the chimera assay. For example, maturing oocytes (those irradiated between 1 and 6 weeks prior to mating) do not show a cell proliferative disadvantage in the chimera assay (Table 1), while these cells were the most mutable using the seven-specific-locus test [1]. These findings suggest that the chimera assay is either so sensitive that it is able to resolve responses that are unresolvable using the other assays or involves diffferent mechanisms/targets than the other assays.

The extraordinary sensitivity of the chimera assay has been demonstrated previously both in the male (0.01-Gy-range [6]) and *in vitro* [5,9] and has prompted questions concerning the nature of the radiosensitive target in such an unusually sensitive system. That is, if the target is non-nuclear (i.e., not genetic), as is the case for similarly sensitive immature oocytes of the mouse, this effect may only be transient and thus not of concern for transmission to the offspring. However, if the observed effect is due to genetic damage, this highly sensitive assay may indeed be useful for low-level screening of genotoxic agents.

Table 1
Summary of proliferation ratios for chimeras from oocytes γ-irradiated *in vivo*.

Dose (Gy)	Post-irradiation week mated 6	7	8	12
		Experiment 1		
0	0.50±.009 (45)	0.51±.006 (26)	--	--
0.15	0.50±.012 (32)	0.47±.011* (30)	--	--
		Experiment 2		
0	0.49±.009 (33)	0.50±.008 (28)	0.50±.013 (23)	0.49±.009 (28)
0.15	0.50±.013 (29)	0.46±.010* (32)	0.48±.013 (23)	0.51±.009 (22)
		Experiment 3		
0	0.50±.015 (21)	0.51±.009 (29)	0.50±.017 (23)	0.52±.025 (13)
0.15	0.51±.010 (24)	0.45±.015** (27)	0.51±.014 (18)	0.53±.010 (16)

*p <.05; **p <.005; Mean±SEM; number of chimeras in parenthesis.

Studies to explore whether the target for reduced cell proliferation in early embryos is nuclear or extranuclear are underway using the *in vitro* chimera assay. In these studies, 4- or 8-cell embryos are irradiated *in vitro* and aggregate chimeras made using one treated and one untreated embryo. Our approach is to compare cell proliferative disadvantage following incorporation of ^{3}H-Tdr into DNA (nuclear dose), incubation of embryos in medium containing ^{3}HOH (uniform dose), or irradiation with acute gamma rays (also uniform dose). Results for ^{3}H-Tdr, ^{3}HOH, and ^{137}Cs gamma rays indicate similar responses for the same dose to the nucleus. These results would be expected if the radiosensitive target(s) were nuclear. In contrast, if the target(s) were actually non-nuclear (e.g., the plasma membrane), gamma rays and ^{3}HOH should be much more effective than ^{3}H-Tdr. This is because only a small fraction of the beta rays from tritium confined to the nucleus can actually reach cytoplasmic organelles due to their short range—the plasma membrane would receive only ~1% of the dose to the nucleus from tritium incorporated into DNA [10].

Our results also demonstrate the extraordinary power of this assay to measure effects at very low doses. Significant decreases in proliferation ratios are observed at ^{3}H-Tdr doses to the nucleus of only 0.003 Gy!

In summary, the dose of 0.15 Gy of gamma rays, which resulted in reduced proliferation of embryos from female mice irradiated seven weeks prior to mating, is a factor of at least three lower than the low-LET detection limits for dominant lethals and specific-locus mutations. These results also provide the third study that now has demonstrated transmitted genetic effects from irradiated mouse immature oocytes; the previous two studies have been reported elsewhere [4,11]. Because the chimera assay *in vitro* appears to be measuring nuclear (DNA) damage, the diminished proliferation ratios measured following *in vivo* exposure may also (by inference) result from damage to DNA and thus could potentially transmit mutations to the offspring. Interestingly, the "window" of high sensitivity detected at 7 weeks postexposure corresponds to the time just prior to the transition from resting immature oocytes to growing follicles. This sensitive window has not been observed previously. Future work will explore whether a transmitted proliferation disadvantage would be detectable following higher doses to mouse oocytes at other stages of development and whether the radiosensitive target for the transmitted effect in oocytes is nuclear or extranuclear.

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