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ORIGINAL PAPER

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The relative biological effectiveness of densely ionizing heavy-ion radiation for inducing ocular cataracts in wild type versus mice heterozygous for the *ATM* gene

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Abstract The accelerated appearance of ocular cataracts at younger ages has been recorded in both astronauts and airline pilots, and is usually attributed to high-energy heavy ions in galactic cosmic ray radiation. We have previously shown that high-LET 1-GeV/nucleon ^{56}Fe ions are significantly more effective than X-rays in producing cataracts in mice. We have also shown that mice haploinsufficient for *ATM* develop cataracts earlier than wild-type animals, when exposed to either low-LET X-rays or high-LET ^{56}Fe ions. In this paper we derive quantitative estimates for the relative biological effectiveness (RBE) of high energy ^{56}Fe ions compared with X-rays, both for wild type and for mice haploinsufficient for *ATM*. There is a clear trend toward higher RBE's in haploinsufficient animals, both for low- and high-grade cataracts. Haploinsufficiency for *ATM* results in an enhanced sensitivity to X-rays compared with the wild type, and this enhancement appears even larger after exposure to high-LET heavy ions.

Introduction

Increased risks of ocular cataracts are one of the medical legacies of space flight that has been observed in astronauts, as described by Cucinotta et al. [1]. In addition, there have been reports of an increased risk of cataract in airline pilots [2]. While cataracts are not, in themselves, life-threatening and can be readily managed surgically, they nevertheless represent a biomarker of accumulated cellular injury.

Cataracts are rare at younger ages, but common in old age, in many animals as well as man. However, in individuals that have been exposed to agents that cause damage to the developing lens fibers, such as ionizing radiation [3], the appearance of a cataract is typically accelerated to younger ages, in a dose-dependent manner [4].

Both astronauts and airline pilots are exposed to comparatively low doses of ionizing radiations, which consist of different mixtures of sparsely ionizing radiation such as gamma rays and protons, and densely ionizing radiations such as neutrons and heavy ions. In the study reported by Cucinotta et al. [1] of about 300 astronauts, an increased risk of cataractogenesis was apparent from missions that involved quite low doses of densely ionizing heavy ions. In animal models, low doses of such densely ionizing radiations have been shown to be highly effective for cataractogenesis [5, 6], thus an increased cataract risk in astronauts and airline pilots is not unexpected.

In this study, we address a central question of whether the phenomenon of accelerated cataract induction occurs randomly in different individuals, or whether these cataracts are preferentially occurring in genetically predisposed individuals, i.e., in radiosensitive sub-populations.

The most studied, and certainly the most prevalent radiosensitive group that is currently known is the subpopulation of AT (ataxia telangiectasia) heterozygotes [7]. Ataxia telangiectasia has been shown to be associated with increased radiation sensitivity when mutations in both alleles of the *ATM* gene are present [8]. In addition to elevated radiation sensitivity, individuals homozygous for *ATM* express varied neuropathies, immunological anomalies, and cancer predisposition [9]. More common are individuals with mutations in only

Dedicated to the memory of Professor Basil V. Worgul, who passed away in January 2006, much missed by all his colleagues.

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one allele ($ATM^{+/-}$), who comprise about 1–3% of western populations [10, 11]. Although phenotypically indistinguishable from the general population, individuals heterozygous for *ATM* have an increased risk of developing cancer [12, 13], and there have been suggestions that this population is at increased risk for radiation-induced cancer [14].

It is now clear that the ATM protein is the major kinase that initiates DNA damage signal transduction responses following exposure to ionizing radiation [15]. For example, cell lines defective for *ATM* display cell cycle checkpoint defects [16] and increased radiosensitivity [17], while in vivo, there are reports suggesting that radiotherapy patients who are heterozygous for the *ATM* gene are at greater risk of developing late normal-tissue damage [18, 19].

We have recently published data for cataractogenesis in mice that are wild type or haploinsufficient ($ATM^{+/-}$) for the *ATM* gene, exposed either to low-LET X-rays [20, 21] or to high-LET 1 GeV/nucleon iron (^{56}Fe) ions [21]. In the case of X-rays, a range of doses were used and dose-dependent prevalence curves were obtained for the induction of various cataracts grades, from early onset (grade 0.5) to vision impairing (grade 2.5). The results [20, 21] showed that, for high X-ray doses, haploinsufficiency for *ATM* resulted in cataracts developing some 10–15 weeks earlier than in the corresponding wild-type animals; at low X-ray doses, cataracts appeared in AT heterozygotes, whereas none appeared (at least until old age) in the wild type animals.

For the iron-ion irradiations [21], only one radiation dose was used, namely 0.325 Gy, which corresponds to approximately one track traversing each irradiated cell. As with the X-ray results, mice that were haploinsufficient for *ATM* ($ATM^{+/-}$) developed cataracts earlier than their wild type littermates; in addition, at this dose, *ATM* heterozygotes developed grade 2.5 (vision impairing) cataracts, while the wild type animals did not.

In our earlier report [21], because of the experimental design used, we did not estimate the relative biological effectiveness (RBE) of the heavy ions for cataractogenesis in the wild type versus $ATM^{+/-}$ animals. In the new analysis described here, we are able to estimate the RBE for iron-ion induced cataractogenesis, for cataracts of varying grade. RBEs are estimated and compared for mice that are haplo-insufficient for *ATM*, and for the corresponding wild type mice.

Materials and methods

Details of the irradiation methods and the cataract measurement techniques are given in the earlier papers [20, 21]. Briefly, $ATM^{+/+}$ and $ATM^{+/-}$ mice (10 per group) were anesthetized and irradiated at Columbia University with one of five doses of 250-kVp X-rays (0.5, 1, 2, 4, or 8 Gy), or with a dose of 0.325 Gy of 1 GeV/amu iron (^{56}Fe) ions (stopping power ~148 keV/μm), at the NASA Space Radiation Laboratory (NSRL) at Brookhaven National Laboratory. The 0.325 Gy iron-ion dose corresponds to a mean of approximately one iron-ion passing through each irradiated cell nucleus. A full description of the dosimetry methods and characterization of the Fe beam has been published previously [22].

After irradiation, the animal eyes were examined three times each week for up to 60 weeks. Eyes were dilated prior to slit lamp examination, and cataracts were scored using the quantitative cataract grading system devised by Merriam and Focht [23], which assigns cataract grades from early onset (grade 0.5), through definitive cataract (grade 1.0), to vision impairing (grades 2.0 and 2.5).

These data have been analyzed to produce grade-dependent prevalences, i.e., the probability, corrected for competing risks, that an animal will develop a cataract of a particular grade by a given time, as a function of radiation type, dose, and animal type ($ATM^{+/+}$ or $ATM^{+/-}$). Further details of the analysis technique have been given elsewhere [24]. In brief, the Kaplan–Meier technique [25] was used to obtain nonparametric

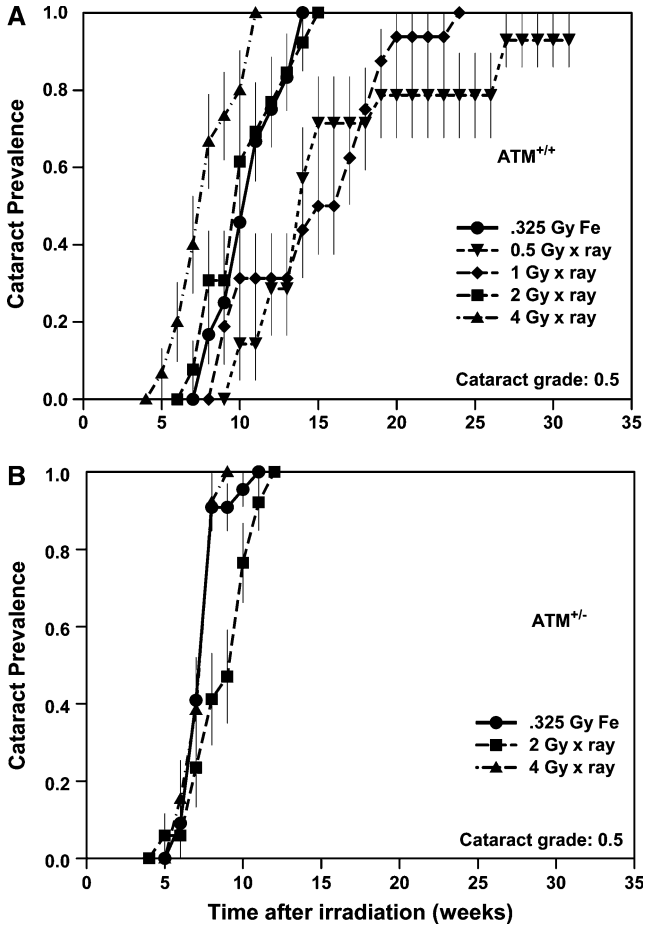

Fig. 1 Prevalence of grade 0.5 cataracts, as a function of time since exposure to single iron-ion dose (0.325 Gy), as well a range of X-ray doses, for **a** wild-type mice ($ATM^{+/+}$), and **b** $ATM^{+/-}$ mice, i.e., haploinsufficient for *ATM*

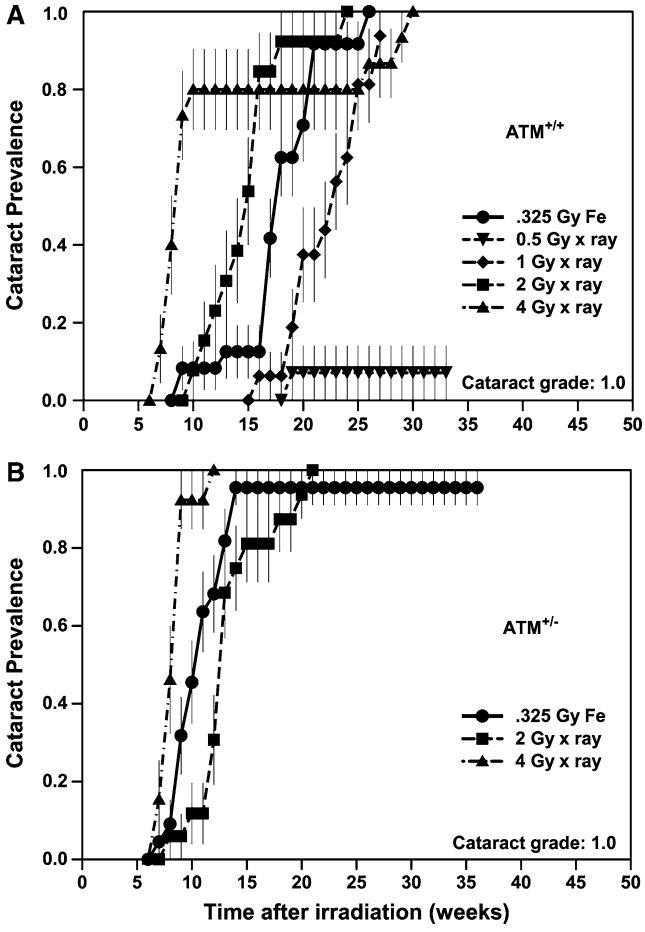

Fig. 2 As Fig. 1, for grade 1.0 cataracts

maximum-likelihood estimates of the grade-specific cataract prevalence as a function of time after irradiation, corrected for competing risks; estimates of the standard error were made using Greenwood's formula [26].

Estimation of relative biological effectiveness

The relative biological effectiveness (RBE) is defined as the ratio of doses of X-rays to the test radiation (in this case iron-ions) to produce the same biological effect, in this case the same grade of cataract. Because of the significant resources required for these experiments, we used a limited number of radiation doses; in particular, one dose of iron-ions (0.325 Gy) and five doses of X-rays (0.5, 1, 2, 4, and 8 Gy) were used. Because the RBE requires estimating the specific X-ray dose that produces the same level of cataractogenesis as the 0.325 Gy iron-ion dose, this experimental design does not directly permit point estimates of the RBE, as opposed to a confidence interval estimate.

However, because the prevalence data are generally monotonically increasing with increasing dose, it is possible to generate a bivariate interpolation of the X-ray prevalence data (i.e., of the prevalence as a function of X-ray dose and time post exposure). This was done using the algorithm described by Akima [27]. The interpolating function is a fifth degree polynomial in dose and time defined in each triangular cell which has projections of three data points in the dose-time plane as its vertices. Given this interpolation of the X-ray prevalence data, it is possible to generate point estimates (though not confidence intervals) of the RBEs.

Specifically, having the interpolated prevalence data available at any X-ray dose, we are able to find the X-ray dose which produces the pattern of time- and grade-dependent prevalence which is "most similar" to that produced by 0.325 Gy of iron-ions. We interpret "most similar" in the least squares sense, i.e., we search for the X-ray dose that minimizes the quantity

$$\sum_T \left(\frac{P_x(D,t) - P_{\mathrm{Fe}}(0.325\ \mathrm{Gy}, t)}{P_{\mathrm{Fe}}(0.325\ \mathrm{Gy}, t)} \right)^2, \qquad (1)$$

where $P_A(D,t)$ is the cataract prevalence for a given grade and animal type ($ATM^{+/+}$ or $ATM^{+/-}$) as a function of the dose (D) of radiation type A, at a time t after exposure; here the summation covers all the

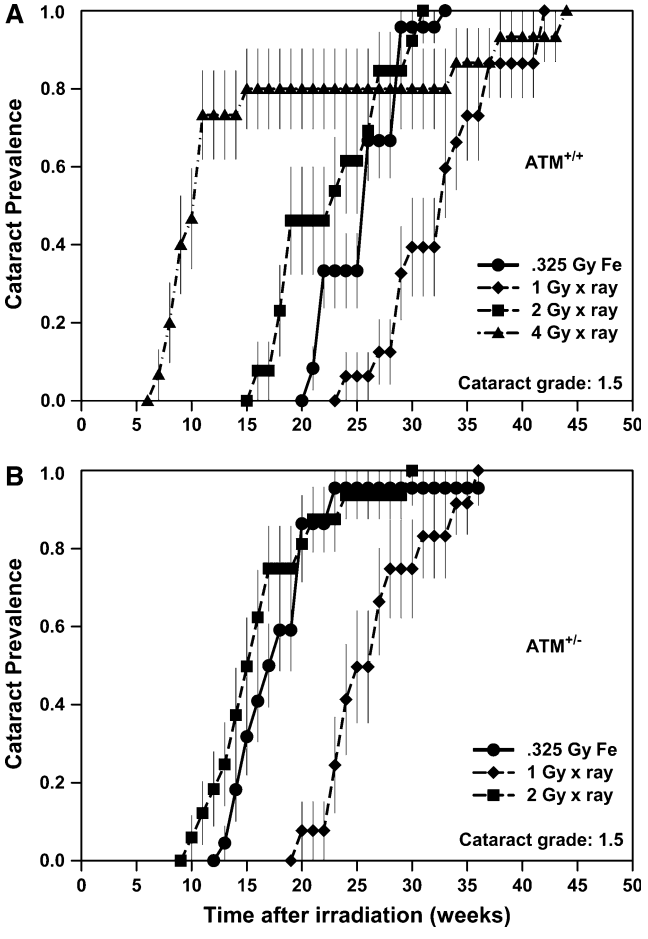

Fig. 3 As Fig. 1, for grade 1.5 cataracts

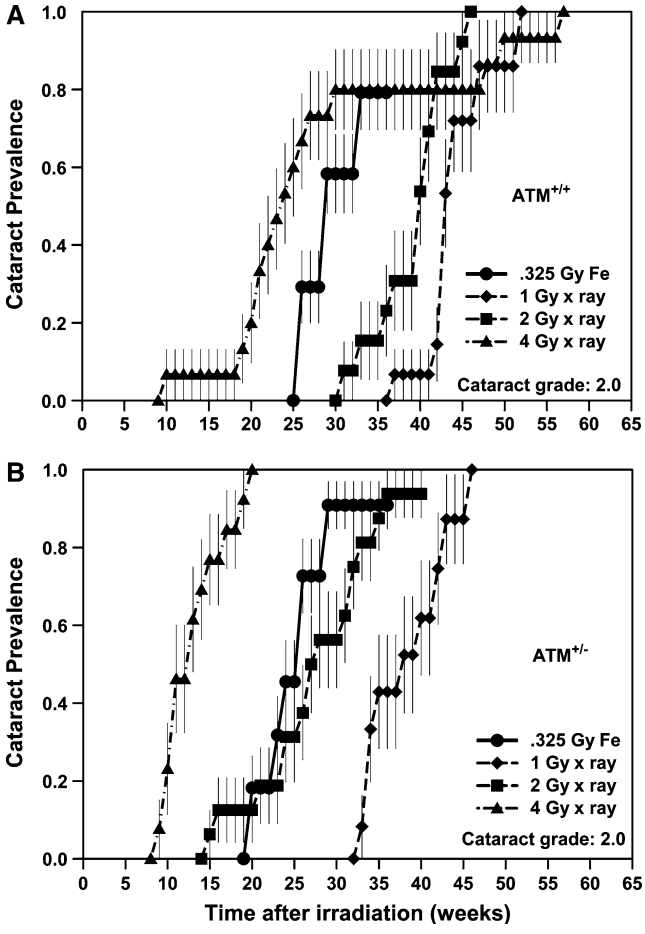

Fig. 4 As Fig. 1, for grade 2.0 cataracts

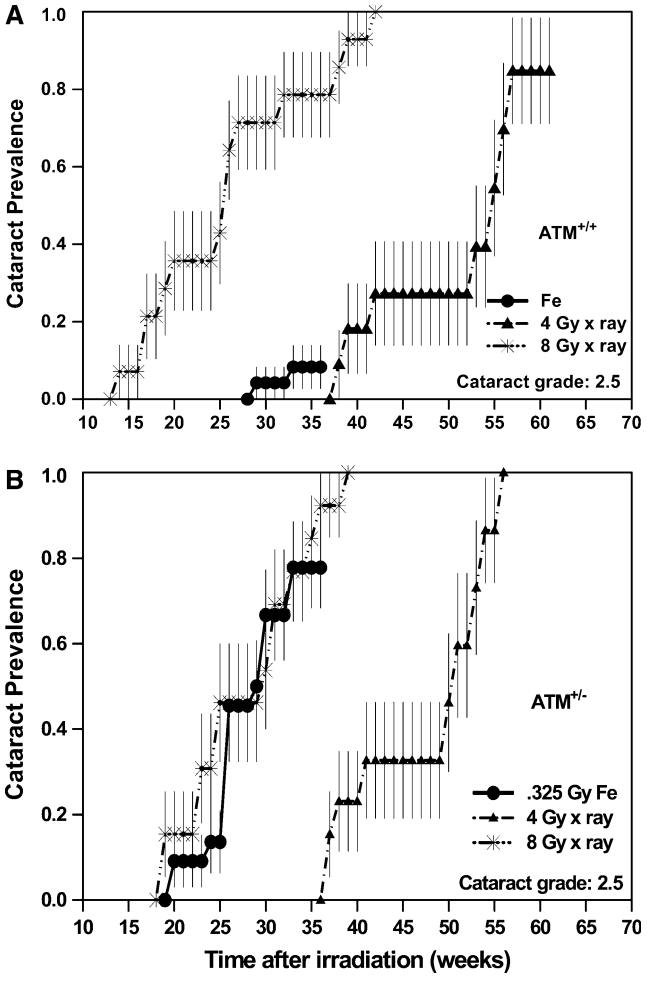

Fig. 5 As Fig. 1, for grade 2.5 cataracts

measured time points, T, in the particular iron-ion prevalence data set (grade and animal type) being analyzed. The X-ray dose corresponding to the minimum of this quantity was found by systematically calculating the quantity as a function of X-ray dose, in 0.02 Gy dose increments.

Results

The grade-dependent prevalence data from this work are shown in Figs. 1, 2, 3, 4, 5, and an example of the interpolated X-ray induced prevalence data is shown in Fig. 6. Cataract prevalence data in unirradiated controls have been previously published [21]. Based on these data, and using Eq. 1, the estimated grade-dependent RBE values are shown in Fig. 7; the RBE values for the $ATM^{+/-}$ haploinsufficient animals were somewhat higher than those for the wild type, ranging from 5 to 24. However, because of the nature of the analysis performed, it was not possible to extract RBE confidence limits from the data analysis.

For the wild-type animals, the estimated RBE values of the iron-ions are in the range of 5–15. These RBE values, for 0.325 Gy of 148 keV/μm iron ions, are

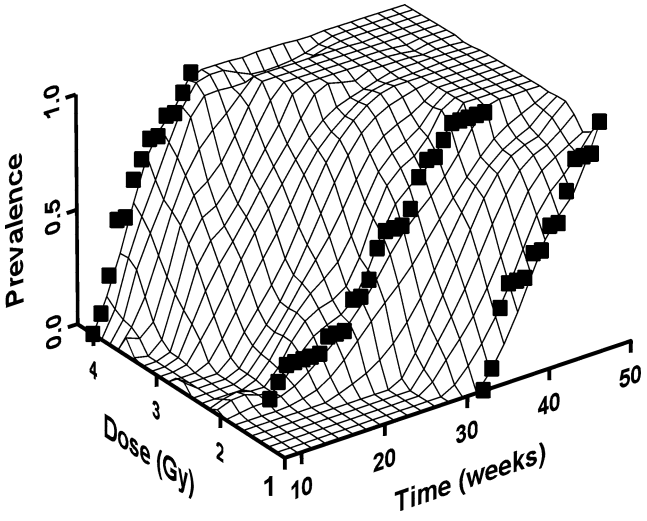

Fig. 6 Example of bivariate interpolation of X-ray prevalence data. In this case the data are for X-ray induced grade 2.0 cataracts in $ATM^{+/-}$ mice. Prevalence data are shown as *points*, and the bivariate interpolated surface is shown as *lines*

consistent with earlier measurements (see Fig. 8) for heavy-ion induced cataractogenesis in wild-type animals, by doses including 0.25 and 0.5 Gy of 88 keV/μm argon ions and 190 keV/μm iron ions [24]. In these earlier studies, only confidence bands for the RBE could be estimated, and the upper and lower 90% confidence limits of the RBEs for the wild-type animals, 35 weeks after exposure, ranged from 8 to 30 at 0.25 Gy, and from 4 to 12 at 0.5 Gy (see Fig. 8).

The current RBE estimates for the wild type animals are also consistent with earlier results in other animal models: Riley et al. [28] reported irradiations of Sprague–Dawley rats with 0.6 GeV/amu iron-ion doses from 0.1 to 2 Gy; they estimated RBE's, based, as is the current work, on decreased latent periods for cataract induction, of about 7.4 for doses from 0.1 to 0.5 Gy. Similarly, Lett et al. [29] reported an RBE of 4–6 for stationary cataract induction in rabbits irradiated with 0.5–5 Gy of 0.46 GeV/amu iron-ions.

Conclusions

It is clear from human studies that ocular cataracts, accelerated to younger ages, are a consequence of exposure to a whole range of damaging agents, including ionizing radiations. It is equally clear that high LET particles are much more effective than low LET X-rays, and that this probably accounts for the reported cataracts in astronauts.

The availability of genetically engineered mice has made it possible to show that animals haploinsufficient for ATM develop cataracts earlier than their wild-type litter-mates, thus, demonstrating the possibility of a radiosensitive sub-group in the human population since 1–3% of humans are ATM heterozygotes. Other low prevalence, high penetrance candidate genes involved in DNA repair and/or check-point control (including BRCA1 and RAD9) are currently under study, which might expand the radiosensitive sub-population.

The present study indicates a clear trend toward higher RBE's for heavy ions in the haploinsufficient animals, both for low and high grade cataracts. Thus, while haploinsufficiency for *ATM* results in an enhanced sensitivity to X-rays compared with the wild type [20], this enhancement appears even larger for exposure to high-LET heavy ions.

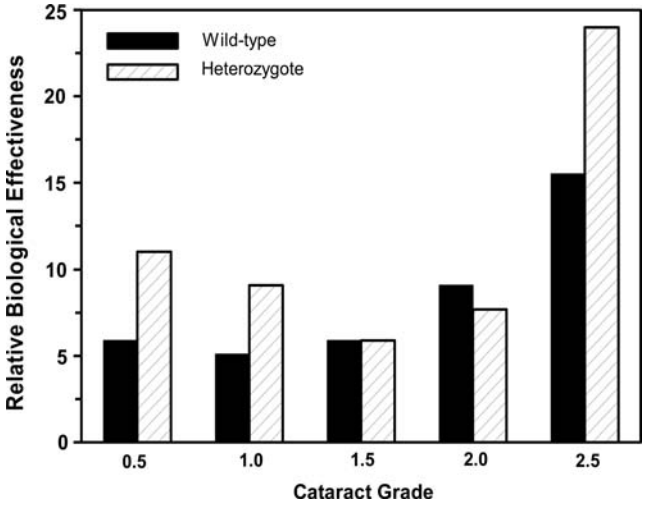

Fig. 7 Estimated grade-dependent RBE values for cataract induction by 0.325 Gy of 148 keV/μm iron ions. *Solid bars*: wild type mice ($ATM^{+/+}$), *Dashed bars*: animals ($ATM^{+/-}$) haploinsufficient for *ATM*

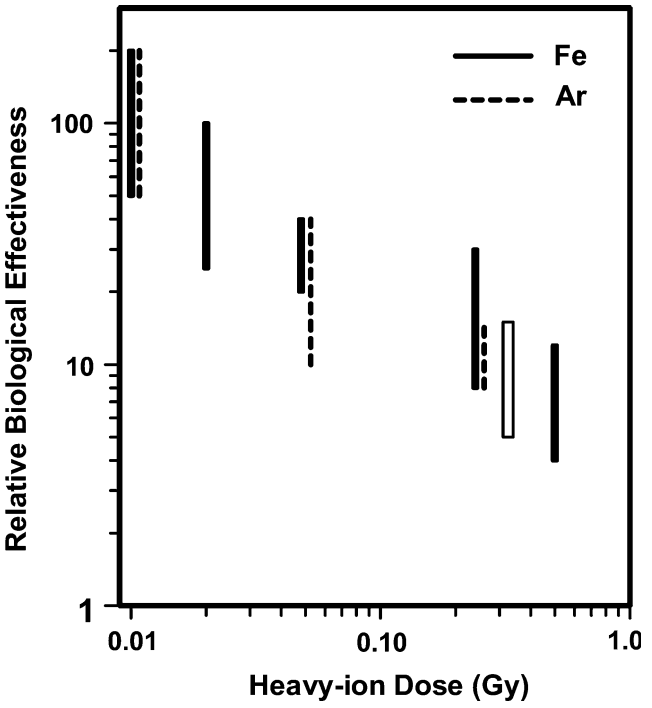

Fig. 8 RBE for heavy-ion induced cataract induction in wild-type rodents. The *open symbol* represents the range of RBE's in wild type animals from the current study with 148 keV/μm iron-ions. The remaining data, taken from Refs. [5, 23], represent grade-independent RBE regions for different doses which were not excluded at the 90% confidence level; *solid symbols* refer to 190 keV/μm iron-ions, *dashed symbols* to 88 keV/μm argon ions, both 35 weeks post irradiation

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