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Genetic susceptibility to radiation

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Abstract

In the context of space radiation, it is important to know whether the human population includes genetically predisposed radiosensitive subsets. One possibility is that haploinsufficiency for ATM confers radiosensitivity, and this defect involves 1–3% of the population. Using knock-out mice we chose to study cataractogenesis in the lens and oncogenic transformation in mouse embryo fibroblasts to assay for effects of ATM deficiency. Radiation induced cataracts appeared earlier in the heterozygous versus wild-type animals following exposure to either gamma rays or 1 GeV/nucleon iron ions. In addition, it was found that embryo fibroblasts of Atm heterozygotes showed an increased incidence of oncogenic transformation compared with their normal litter-matched counterparts. From these data we suggest that Ataxia Telangiectasia heterozygotes could indeed represent a societally significant radiosensitive subpopulation.

Knock-out mice are now available for other genes including BRCA1 and 2, and Mrad9. An exciting possibility is the creation of double heterozygotes for pairs of mutated genes that function in the same signal transduction pathway, and consequently confer even greater radiosensitivity.

Keywords: Heavy ions; Cataracts; Transformation; Genetic susceptibility

1. Introduction

There are a number of areas of human endeavour where the implicit assumption is made that the human population is uniform in its radiosensitivity, ie in its response to radiation, except for a few individuals such as AT homozygotes who are exquisitely sensitive to radiation, but easily identified by their clinical symptoms (Shiloh, 2001). This includes ground-based radiation protection standards, radiotherapy protocols for cancer patients which are seldom customized to the individual; and last but not least, radiation protection standards for space flight.

There are a number of hints from human studies that the assumption of uniform radiosensitivity is incorrect. For example, there is the troubling fact that a few percent of radiotherapy patients suffer severe late effects quite out of proportion to the experience of the majority (Hall et al., 1998). There is also the case of a small group of early onset breast cancer patients in the women who survived the A-bombs in WWII, the genetic basis of which has never been sorted out (Tokunaga et al., 1994).

In the context of Space Radiation Risk Assessment, the existence of an unidentified radiosensitive sub-population would have two consequences. First, it might be considered unethical to put a radiosensitive individual into a situation where they might receive a large dose of radiation because of the possible severe clinical response. Second, the existence of a radiosensitive subpopulation in an epidemiological study would tend to distort the shape of the dose–response relationship, thereby rendering a linear extrapolation from high to low doses invalid. This latter point is illustrated in

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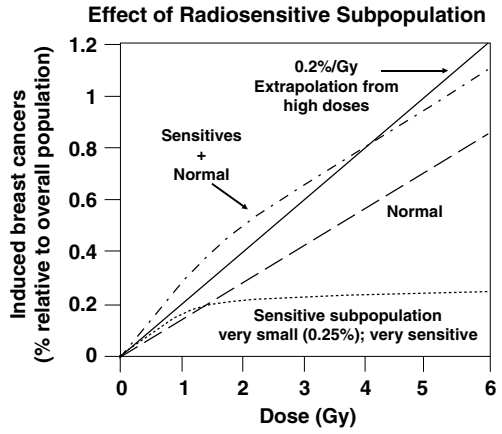

Fig. 1. Illustrating how a small very radiosensitive sub-population can influence the shape of the dose–response relationship. Suppose the dose response relationship for cancer for the bulk of the population (the "normal" population) is a linear function of dose. The addition of a small radiosensitive subpopulation would cause the overall dose response relationship to be curved. As a consequence, a linear extrapolation from high to low doses would underestimate risks at low doses. (Courtesy of Dr. David Brenner.)

Fig. 1, where the possibility is exaggerated to make the point clear.

Here we consider the significance of a very small subpopulation (in this case 1/4%) of very sensitive individuals. This leads to the dose–response curve labeled 'sensitives', which quickly saturates with dose, while the dose–response for the "normal" population (which will not saturate at relevant doses) is drawn as the long-dashed line, chosen such that the total (solid line, normal + sensitives) dose–response curve will be similar to the 0.2%/Gy linear dose response which is inferred from epidemiological data for breast cancer over the ~0.5–6 Gy range. However, while the "true" total curve and the 0.2%/Gy linear dose–response relationship are by definition, quite similar over the range in which epidemiological studies can be done, at lower doses this will not be the case, in that the significance of the sensitive subpopulation, which will not be reflected in the 0.2%Gy curve, will be much larger.

For example, at 6 cGy, the example in Fig. 1 would predict lifetime risks of $2.3/10^4$ from the entire population (normal + sensitives), to which the sensitives contribute 1.4 and the "normals" 0.9, while the linear dose–response curve (which is largely based on the cancer induction rate in the higher dose "normal" population), predicts a total value of 1.2.

In summary, even a very small subpopulation, if they were highly radiosensitive, would significantly distort a linear extrapolation of practical epidemiological data down to low doses. This is because "evaluated" linear dose response curves are currently necessarily based on fitting higher-dose incidence data, where the sensitive subpopulation will have only a small effect. However at low doses, below the realm of classical radiation epidemiology, the effect of a sensitive subpopulation may become significant, or even dominate (as in the above example), and this would not be reflected in a linear extrapolation from higher doses.

In the example given above, about 60% of the low-dose radiation-induced breast cancers comes from a very small sensitive subpopulation – and would not be adequately predicted by an extrapolation from data at higher doses. The example is relatively extreme, in that the assumed Do for the sensitive population is low, just to illustrate the point. More realistic values would produce corresponding lesser problems in high-to-low dose extrapolations.

1.1. Genes involved in radiosensitivity

It may be possible, eventually, to identify families of genes that may cooperate to modify radiosensitivity by the use of gene arrays. This approach has not to date proved to be singularly successful, but it may prove to be the ultimate choice. A simpler strategy is to focus attention on genes that occupy a central position and play a pivotal role in DNA repair and/or checkpoint control, and try to establish their importance in controlling radiosensitivity. An obvious contender is the ATM gene. Its role in repair and checkpoint functions is illustrated in Fig. 2. Ataxia telangiectasia (A-T) is a well known but relatively rare autosomal recessive disorder, which has been shown to be associated with a greatly increased radiation sensitivity when mutations in both alleles of the *ATM* (A-T mutated) gene are present. In addition to elevated radiation sensitivity, individuals homozygous for ATM express varied neuropathies, immunological anomalies, and cancer predisposition (Tokunaga et al., 1994). The more important question

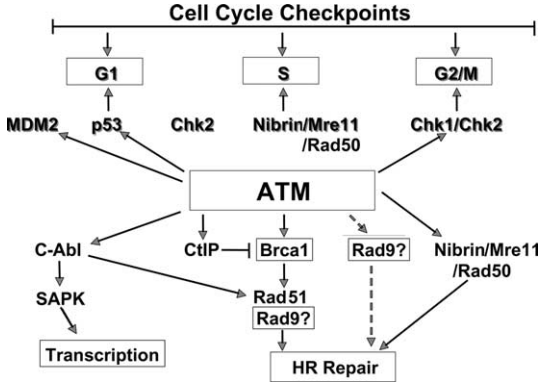

Fig. 2. The central role of ATM the in cellular response to DSBs. ATM is involved in regulating multiple cell cycle checkpoints, possibly through phosphorylation of different targets at different stages of the cell cycle. ATM also signals to repair machinery through its interaction with and phosphorylation of targets implicated in DNA repair. It is also likely that ATM controls the transcription of stress response genes through its interaction with BRCA1 and c-Abl. (Adapted from Khanna et al., Cell Death Differ. 8:1052–1065, 2001.)

is, are individuals who are heterozygous for mutations in ATM, and appear clinically normal, radio sensitive! This is important because 1–3% of the US population fall into this category (Swift et al., 1987).

Several years ago a study was performed to ascertain whether it was possible to account for the 5% or so of radiotherapy patients who suffer severe late effects in terms of their status as AT heterozygotes (Hall et al., 1998). The ATM gene was sequenced in 17 patients who suffered severe late effects; 4 significant mutations were found. These results indicate that late effect patients are rich in AT heterozygotes, but clearly mutations in this gene are not the whole story.

1.2. Atm knock-out mice

Human studies are notoriously difficult and so we turned our attention to knock-out mice, where the *Atm* gene is disrupted by inserting a neo cassette, with the consequence that there was no presence of full-length or truncated protein in the knock-out animals (Elson et al., 1996). One eye of wild-type, Atm heterozygous and homozygous knockout mice was exposed to graded doses of X-rays at Columbia, or high energy Fe56 ions at the Brookhaven Alternating Gradient Synchrotron. Eyes were examined over the next 18 months and cataract grades scored (Merriam and Focht, 1962). Details of the methodology and data analysis have previously been described (Worgul et al., 2002). Data for cataract prevalence after X-ray exposure are shown in Figs. 3 and 4. The Atm homzygotes all developed cataracts at all radiation doses, but this is not of much interest. At a large dose (4 Gy) the Atm heterozygotes developed vision impairing cataracts (grade 2.0) 10 or more weeks early than the wild-type counterparts. At

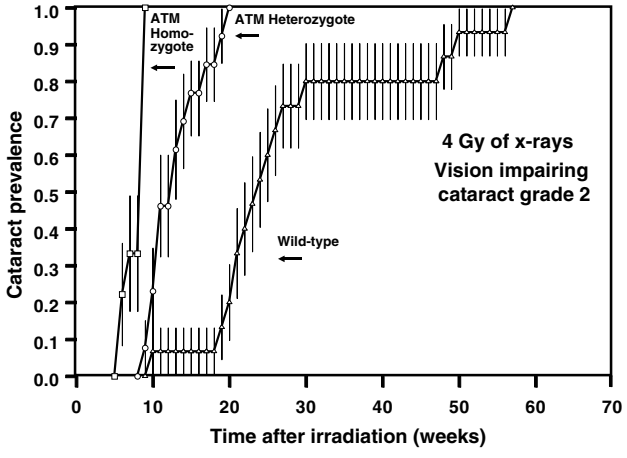

Fig. 3. Prevalence of cataracts of grade 2 (vision impairing) as a function of time after exposure to 4 Gy of X-rays in wild-type mice and in animals homozygous or heterozygous for the ATM gene. The heterozygous animals develop grade 2 (vision impairing) cataracts about 10 weeks earlier than wild-type animals. The vertical bars are standard errors. (Redrawn from Worgul et al., 2002.)

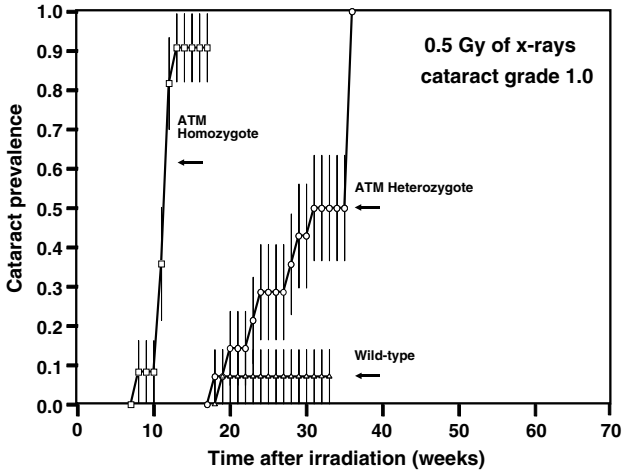

Fig. 4. Cataract prevalence (grade 1) as a function of time after exposure to 0.5 Gy of X-rays in wild-type mice or in animals homozygous or heterozygous for the ATM gene. Note that at this dose, the lowest used in this study, wild-type animals are essentially unaffected, whereas half of the A-T heterozygotes develop a grade 1.0 cataract. The vertical bars are standard errors. (Redrawn from Worgul et al., 2002.)

a lower dose (0.5 Gy) the Atm heterozygotes developed a low grade cataract, while the wild-type did not. We can conclude from this study that

(a) Vision impairing cataracts appear earlier in Atm heterozygotes than in wild-type animals; the acceleration by 10 weeks is an appreciable fraction of the life-span of the animal.
(b) At lower doses, low grade cataracts appear in Atm heterozygotes where none appear in wild-type animals.
(c) The difference in radiation response between Atm heterozygotes and wild-type animals is large and unequivocal in this tissue system, compared to the very small difference in cell survival seen for cells taken from these animals.

Since the function of the ATM controls, among other things, DNA repair and checkpoint controls, we wondered whether the difference seen between wild-type and Atm heterozygotes when exposed to X-rays would be as large or as apparent for a high LET radiation such as a Fe 56 ion. Figs. 5 and 6 show cataract data for animals exposed to 32.5 cGy of high energy heavy ions. Once again cataracts appear earlier in the Atm heterozygotes and the acceleration is similar to that seen for X-rays. Also, at this dose, vision impairing cataracts occur in Atm heterozygotes, but not in wild-type animals.

1.3. Cataracts in astronauts

These data on radiation induced cataracts in mice of different genetic backgrounds assume additional

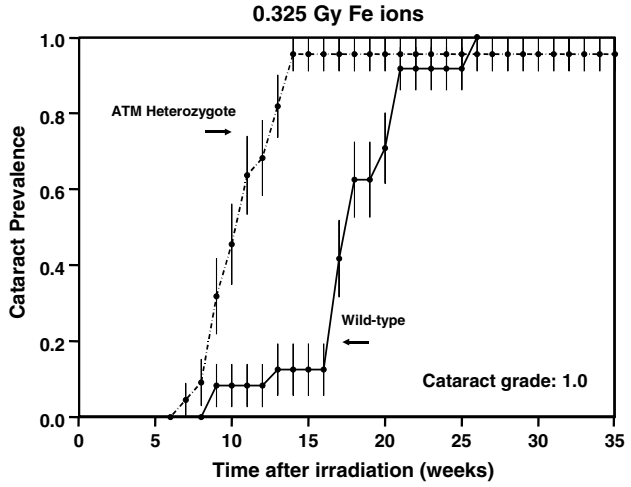

Fig. 5. Prevalence of cataracts (grade 1) as a function if time after exposure to 32.5 cGy of high energy Fe ions in wild-type mice or in animals heterozygous for the ATM gene. Note that the heterozygous animals develop cataracts earlier than their wild-type counterparts.

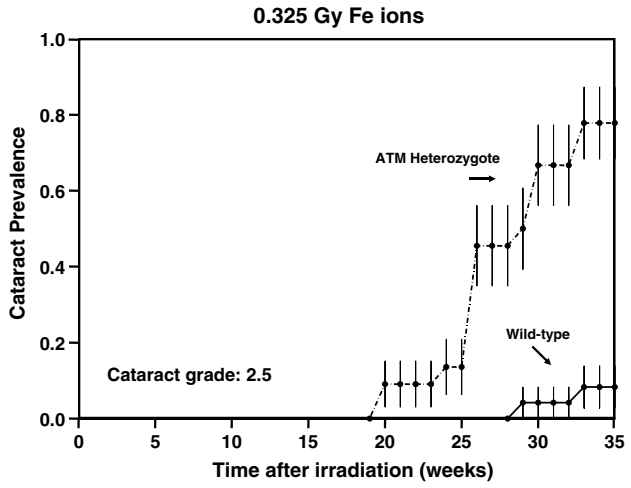

Fig. 6. Prevalence of vision impairing cataracts (grade 2.5) as a function of time after exposure to a dose of 32.5 cGy of high energy Fe ions in wild-type mice of in animals heterozygous for the ATM gene. Note that at this dose, that corresponds to about one particle track per cell nucleus, few wild-type animals develop a vision impairing cataracts, compared with 80% of the heterozygotes.

significance because of the observation of an increased risk of cataracts in astronauts with higher lens doses (>8 mSv) of space radiation relative to astronauts with lower lens doses. (Cucinotta et al., 2001) Of even more interest is the fact that 35 of the 39 cases of cataracts after space flight occurred in astronauts who participated in lunar missions or high inclination shuttle flights, possibly because of the higher flux of high energy heavy ions in these situations. The vast majority of the recorded cataracts involved opacities of the cortical and/or posterior subcapsular variety, which is highly and definably characteristic of radiation as the causative agent. Overall, 3 of the 295 astronauts followed developed vision-impairing cataracts that required surgery relatively early in life, even though the accumulated doses were quite low. It is interesting to speculate that this may indicate a genetically predisposed sensitivity in these individuals.

1.4. Studies with oncogenic transformation in atm and wild-type animals

As a surrogate for carcinogenesis, we studied oncogenic transformation in embryo fibroblasts from Atm heterozygotes and wild-type animals (Smilenov et al., 2001).

Atm heterozygous animals were mated and mid-term embryos harvested and genotyped. It was often possible to obtain embryos in all three categories (wild-type, homozygous and heterozygous) from the same litter. Cell cultures were established from each embryo, sparsely seeded cells exposed to 2 Gy of γ-rays, and scored for cell survival and for the appearance of morphologically transformed foci 2 weeks later. Whereas nontransformed normal colonies consist of rounded, contact-inhibited, monolayers of cells, transformed colonies contain elongated cells in parallel bundles that typically criss-cross each other and do not exhibit contact inhibition; these transformed colonies appear dark blue when stained with Giemsa in comparison with normal contact-inhibited cells because of the presence of multiple layers of abnormal cells. The transformed cells grow and demonstrate anchorage independence in semisolid agar and also produce tumors when injected into athymic nude mice, thus serving as a reliable marker of neoplastic transformation. (When foci identified as transformed by their morphology are expanded in culture, 10^6 cells injected into nude mice form fibrosarcomas in 2–3 weeks in 80% of animals.)

Litter-matched experiments were performed as outlined above to investigate the influence of Atm heterozygosity on radiation-induced oncogenic transformation of MEFs. Experimental details have been published previously (Smilenov et al., 2001). A total of 13 intralitter comparisons were made between normal and A-T heterozygote embryos. Yields of transformed clones were measured both for zero-dose exposure and for exposure to a γ-ray dose of 2 Gy. To directly compare the sensitivities to radiation oncogenesis of the wild-type MEFs with the corresponding ATM heterozygous cells, only litter-matched comparisons were made between the radiation sensitivities of Atm wild-type and heterozygous MEFs. We define the ROR (Relative Oncogenesis Ratio) as the yield of transformed clones per surviving ATM heterozygous MEFs exposed to a dose of 2 Gy relative to the yield of transformed clones per corre-

Table 1
Litter-matched comparisons of radiation oncogenesis between heterozygous and normal wild-type MEFs

	NIH mice	Harvard mice	All mice
Zelen test for homogeneity of ROR[a] (99% confidence limits of P)	$P = 0.68$ (0.68, 0.68)	$P = 0.054$ (0.051, 0.058)	$P = 0.19$ (0.18, 0.19)
Estimated ROR (95% CI) (two-sided P)	1.48 (0.65, 3.51) ($P = 0.35$)	1.89 (1.08, 3.42) ($P = 0.024$)	1.74 (1.11, 2.80) ($P = 0.016$)

To directly compare the sensitivities to radiation oncogenesis of the wild-type MEFs with the corresponding *ATM* heterozygous cells, stratified 2×2 comparisons were used, i.e., only litter-matched comparisons were made between the radiation sensitivities of *ATM* wild-type and heterozygous MEFs. This was done using a Monte-Carlo simulation of Zelen's exact test (Zelan, 1971).

[a] ROR or relative oncogenesis ratio is the yield of transformed clones per surviving ATM heterozygous MEFs exposed to a dose of 2 Gy relative to the yield of transformed clones per corresponding surviving wild-type MEFs also exposed to 2 Gy.

sponding surviving wild-type MEFs also exposed to 2 Gy.

The RORs for the heterozygous versus wild-type MEFs were exactly estimated using standard maximum likelihood techniques (Cox, 1970) and the null hypothesis that the ROR was unity (no difference in sensitivity) subjected to a two-sided test. The results are shown in Table 1.

The ROR (heterozygous versus wild-type) was 1.74 (95% CI, 1.11–2.80) and the null hypothesis (ie no difference) could be rejected ($P = 0.02$), i.e., the Atm heterozygous mice were significantly more sensitive for radiation oncogenesis than were the corresponding wild-type animals by a factor of almost 2.

By contrast, the ROR at 2 Gy for Atm-deficient homozygous mice compared with the normal wild-type was 10.5 (95% DI, 4.4–26.2; $P < 0.001$; 4 litter-matched comparisons made, data not shown).

1.5. Knock out mice available for future studies

In addition to the Atm knock-outs, we have available animals heterozygous for the BRCA 1, the BRCA 2 and the Mrad9 gene. Knockouts for BRCA 1 or 2 are embryonic lethal. Interestingly, we have also created double-heterozygotes for Atm het/Mrad9 het and Atm het/BRCA1 het which appear to develop normally. These combinations were chosen because it is evident from Fig. 2 that these pairs of genes function in the same signal transduction pathways and may therefore amplify their effects on radiosensitivity. In future studies we plan to investigate the influence of these genes on the incidence of ocular cataracts and oncogenic transformation.

Acknowledgements

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