Adaptive and reverse adaptive responses for chromosomal inversions in pKZ1 mouse prostate: hints on mechanism.

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An adaptive response is a response to a stress such as radiation exposure, which results in a lower than expected biological response. We have demonstrated an adaptive response to X-radiation in mouse prostate using the pKZ1 chromosomal inversion assay. pKZ1 mice were treated with a priming dose of 0.001, 0.01, 1 or 10 mGy followed 4 hours later by a 1000 mGy challenge dose. All priming doses caused a similar reduction in inversions relative to the 1000 mGy group, supporting the hypothesis that the adaptive response is the result of an on/off mechanism (Fig 1A). A priming dose of 0.001 mGy is three orders of magnitude lower than the lowest priming dose that has previously been reported to induce an adaptive response for any end-point. The adaptive responses for all priming doses completely protected against the inversions that would have been induced by a single 1000 mGy dose, as well as against a proportion of spontaneous background inversions. We have also performed reverse adaptive response experiments where mice were exposed to a high 1000 mGy dose and then 4 hours later they were exposed to a low dose of 0.001 mGy or 10 mGy (Fig 1B). In both cases an adaptive response for chromosomal inversions was induced. Therefore the low priming dose (given either before or after the high dose) induces a response which can protect from inversion induction within four hours of high dose irradiation.

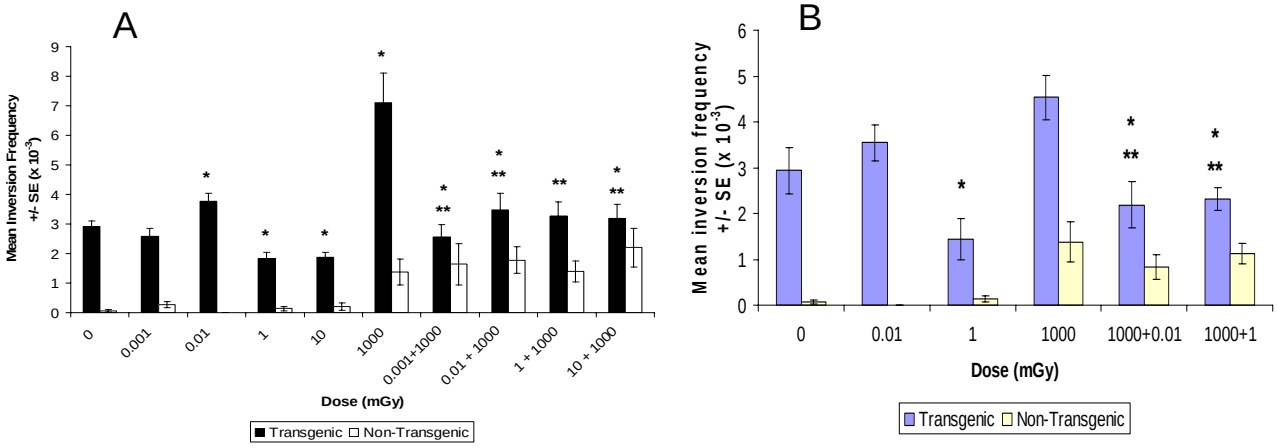

Figure 1. Mean inversion frequency (± SE) in pKZ1 prostate after priming + challenge whole body X-irradiation. $4 \leq n \leq 10$ transgenic mice, and $2 \leq n \leq 5$ non-transgenic mice. *: Significant change in corrected inversion frequency between sham-treated and treated mice. **: Significant change in corrected inversion frequency between priming + challenge and challenge only groups. [$p<0.05$, 2-tailed Mann Whitney U test]. Corrected inversion frequencies were calculated by transgenic minus non-transgenic to correct for non-specific staining.

The distribution of inversions in individual prostatic glandular cross-sections was similar between all priming plus challenge groups and suggested that the chromosomal inversion adaptive response is largely the result of a low dose response (Response L, Fig 2), because high dose induced inversions remained in the presence of an adaptive response (Response H, Fig 2).

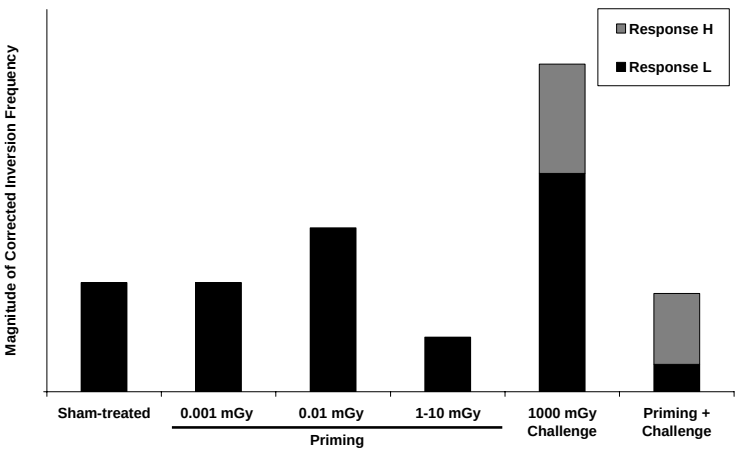

Figure 2. Hypothetical representation of responses to X-irradiation. The type of damage response is based on the distribution of the number of inversions per glandular cross-section. The relative magnitude of response in each treatment group relates to the overall inversion frequency. Low dose radiation damage response (L) refers to inversions which are produced in response to low dose radiation (0–3 inversions per glandular cross-section). Inversions resulting from high dose responses (H) result from high dose radiation exposure (4–7 inversions per glandular cross-section).

The induction of a chromosomal inversion adaptive response in the present study by low doses of radiation either before or after high dose exposure suggests that radioprotective factors do not have to be present at the time of high dose exposure to result in protection from chromosomal inversions. The chemical radioprotector, amifostine, is a free radical scavenger and has been shown to protect from HPRT mutations and radiation induced toxicity both before and after radiation exposure. We performed a preliminary study to determine if amifostine could protect against endogenous inversions in pKZ1 spleen and/or protect against X-radiation induced inversions (Fig 3). Amifostine alone caused a significant reduction in inversion frequency to below the spontaneous frequency (PBS vehicle treated group). This response mimicked the effect previously observed when pKZ1 mice were irradiated with a 1-10 mGy single whole body dose of X-radiation. A dose of 250 mGy X-radiation alone caused an induction in inversions. Amifostine administered three hours after the 250 mGy dose not only resulted in protection from inversion induction but also protected from a proportion of spontaneous inversions.

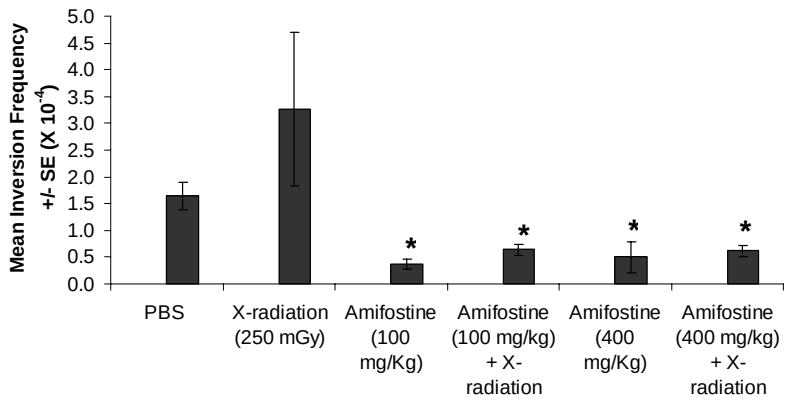

Figure 3. Mean inversion frequency (± SE) in pKZ1 transgenic spleen (T) after either 250 mGy X-radiation alone**,** amifostine alone, or X-radiation followed by amifostine 3 hours later. n=5. No non-specific staining was observed in any of the non-transgenic (NT) controls *: Significant change in inversion frequency between sham-treated and amifostine treated (T – NT) and 250 mGy irradiation plus amifostine treated.

The results presented here suggest that endogenous radioprotective factors are induced at biologically relevant doses and provide hints regarding the mechanism involved in the adaptive response:

- Adaptive responses for inversions in pKZ1 prostate can be induced by single acute doses as low as 0.001 mGy, doses which are relevant to population exposure.
- The magnitude of the adaptive response is similar for a priming dose ranging over three orders of magnitude indicating that the adaptive response involves an on/off mechanism rather than a dose-related mechanism.
- A low dose of radiation can provide protection from chromosomal inversions induced by both high dose exposure and background exposure, either before or after a high dose suggesting that the genes involved in the low dose inversion response and high dose inversion response are different
- The distribution of inversions within prostatic glandular cross-sections suggests that the adaptive response is due largely to a reduction in the low dose induced inversion response rather than the high dose induced inversion response.
- Amifostine, a known radioprotector, causes a reduction in inversion frequency suggesting that a polyamine-like thiol containing free radical scavenger can prevent both spontaneous inversions as well as radiation induced inversions
- Amifostine causes a reduction in inversions even when administered after a radiation dose suggesting that it acts in a similar temporal manner to low dose radiation endogenously produced radioprotectors.

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