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LETTERS TO THE EDITOR

Comments on "Chromosome Intrachanges and Interchanges Detected by Multicolor Banding in Lymphocytes: Searching for Clastogen Signatures in the Human Genome" by Johannes *et al.* (*Radiat. Res.* 161, 540–548, 2004)

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Johannes *et al.* (*1*) recently presented interesting new data on the LET dependence of intrachromosomal aberration yields, which may be compared with earlier theoretical calculations and experimental results. Specifically, Johannes *et al.* reported that their measured ratios of intrachromosomal to interchromosomal aberrations were independent of LET, in contrast to some *in vitro* measurements (*2*), *in vivo* results (*3*), and theoretical expectations (*4, 5*). We suggest there may be two reasons for this discrepancy:

1. For their comparison with low-LET radiation, Johannes *et al.* (*1*) used high-energy 400–500 MeV/nucleon iron ions. All the earlier measurements and calculations related to low-energy α particles or neutrons. The significance here is that, for the high-energy ions used by Johannes *et al.*, there is a large δ-ray (secondary electron) component to the field. For example, Metting *et al.* (*6*) measured the distribution of energy deposition (specific energy) events in a 1.3-μm-diameter target for 600 MeV/nucleon iron ions; here, about 78% of the energy deposition events were from low-LET δ rays rather than high-LET iron ions. This is not the case, for example, for neutrons or α particles emitted by isotopic sources such as plutonium or radon progeny.
2. Johannes *et al.* (*1*) used very different experimental techniques for their studies of high-LET radiation and low-LET radiation. Specifically, the studies at high-LET were undertaken using a calyculin-based premature chromosome condensation (PCC) technique, which assays artificially condensed G_2/M-phase cells; by contrast, the studies at low LET were performed with conventional metaphase techniques after accumulations of Colcemid. Not only are the chromosome yields different using these different techniques (*7*), but the measured distributions of different types of chromosome aberrations have also been shown to be different (*8*).

Thus comparisons between the data for low-LET and high-LET radiation from Johannes *et al.* (*1*) are hard to make because of the different techniques used and because of the mixed field used for the exposures at high LET. It may be, therefore, that the results of Johannes *et al.* are not in disagreement with earlier experimental results, in which the same methodology was used for low- and high-LET exposures, and for which more homogeneous high-LET fields were used.

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We thank Dr. Brenner for his useful comments on our recent paper on mBAND (*1*). We fully agree that there are a number of possible caveats in the search of clastogen signatures in the human genome. Two of such caveats are indeed pointed out by Dr. Brenner: the problem of radiation quality, i.e. the different track structure of particles with different charge at the same LET, and the methods used to harvest and identify chromosomes, in particular the difference between metaphase and prematurely condensed chromosomes. We would like to add a few more comments concerning these issues, which are already very well addressed in Brenner's letter.

1. We fully agree that biomarkers of radiation quality are not dependent simply on LET but on the radiation track structure and microdosimetric energy deposition spectra as well. This has already been shown for cytogenetic end points. For instance, qualitative and quantitative differences were observed in chromosomal aberrations induced by hydrogen or neon ions at the same LET (around 30 keV/μm) in mouse fibroblasts (*2*). Therefore, results with high-energy heavy ions cannot simply be extrapolated to α particles or neutrons. We note, however, that we have also reported data obtained with neutrons (*1*). We did not find any significant changes in the *F* or *G* ratio in lymphocytes exposed to neutrons or X rays, while again a significantly higher fraction of complex-type interchromosomal exchanges was measured.
2. We also agree completely that different results are obtained when scoring metaphase or prematurely condensed chromosomes. We argue, however, that the difference is very small for X rays and much larger for heavy ions, as originally demonstrated in ref. (*3*), also quoted in Brenner's comments. In fact, the yield of X-ray-induced aberrations was similar in prematurely condensed chromosomes and metaphase chromosomes from human lymphocytes, but more aberrations were scored in prematurely condensed chromosomes after exposure to high-LET ^{12}C ions (*3*). More recently, it has been demonstrated clearly that the RBE of heavy ions for the induction of chromosome aberrations in lymphocytes is significantly higher when aberrations are scored in prematurely condensed chromosomes than in metaphase (*4, 5*). These results suggest that metaphase analysis substantially underestimates the cytogenetic damage induced by high-LET radiation, at least when this damage is measured within the first cell cycle after exposure. Biophysical models of the action of charged-particle radiation should therefore be preferentially based on PCC data, or they must consider the impact of exposure on cell cycle delay (*6*). Therefore, we believe that our choice of using prematurely condensed chromosomes for heavy ions and metaphase for X rays is correct and is particularly informative for biophysical models. In passing, we also note that the neutron data were obtained in metaphase cells, and we also did not measure a higher fraction of intrachromosomal exchanges compared to X rays in those samples (*1*).

One major problem in comparing our data with previous results obtained in Giemsa-stained samples (*7*) is the much greater complexity of aberration patterns uncovered by mBAND. Very often intrachromosomal exchanges are associated with interchanges, especially after exposure to high-LET radiation. A detailed classification of these aberrations is needed (*8*), and a common terminology eventually should be used to allow comparison of different results.

In conclusion, we believe that more experiments are necessary to identify potentially useful biomarkers of radiation quality. Even the ratio of complex/simple interchanges, which clearly increases with LET in our experiments as well in many other *in vitro* experiments, does not seem to be able to discriminate low- from high-LET radiation exposures in some cases *in vivo* (*9*), probably because the sensitivity of this parameter depends on the dose. In addition, the issue of persistence of aberrations *in vivo* is decisive when biomarkers are used in retrospective biodosimetry, such as in the recent study of former plutonium workers (*10*).

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