"Radiation-induced genetic changes: potential implications of recent data"

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**Changing paradigms**
The last decade has seen the publication of numerous studies that have challenged some of the paradigms that underly much of our thinking about radiation genetics (reviewed by, eg [1]). Many of these studies have involved tandemly repeated DNA loci (TRDLs) such as microsatellites and expanded simple tandem repeats (ESTRs), minisatellites, and gene duplications in mammalian genomes. Such data as exist indicate that the radiation sensitivity of these sequences for mutation (sequence change) as measured by the doubling dose is comparable to that of other radiation endpoints. However, because some of them have high rates of spontaneous instability, it means that they also have high rates of radiation-induced sequence change. There is also only limited evidence of dose dependence. These more recent data and their implications have been set out at length in a recent review by my colleagues and I [2]; detailed discussion may be found there and I shall here point out only one or two important conclusions.

Firstly, the high absolute radiation sensitivity of some of these sites means that the mutations must be untargeted, that is, most of the effective energy loss events occur elsewhere in the cell and not in the sequence studied. This is in some respects paralleled by the bystander phenomena in which effects are seen in neighbouring cells that have not been irradiated. The bystander effect has been shown for chromosome damage in both mice and humans and has been shown to occur not only in cell cultures but in normal human skin tissue [3]. However, whereas a convincing *in vivo* demonstration of the bystander effect has yet to be made, the untargeted effects at TRDLs are established *in vivo* effects. The untargeted nature of these radiation effects means that one can no longer rely on the linear induction of complex damage due to energy loss events to justify the linear induction of genetic damage in all cells. This should not, however, be taken as an excuse to assume that concave, convex, or other dose response curves are the norm. Some recent analyses with domestic radon have shown very clearly that at low and environmentally relevant doses, the data for lung cancer comply well with a linear dose response model [4], and it seems sensible to continue to regard this as the norm unless there is compelling evidence to the contrary.

The second new challenge to the existing paradigms is the evidence for transgenerational mutagenesis. This has been observed in mice but not (so far) in humans, and the mechanism driving such mutation transmission is not known. By transgenerational mutagenesis is meant the appearance of mutations in the second generation offspring of irradiated parents. (Mutations appearing in the first generation offspring cannot be unambiguously regarded as transgenerational since they may have...
occurred during sperm maturation in the irradiated father.) One is therefore dealing with something that looks very much like a signal transduction process, the dynamics of which may be (expected to be) far from linear with dose. A word of caution is appropriate here. Mouse ESTRs are very different from human minisatellites and the mechanisms for their mutation (though poorly understood) seem to be very different. Nevertheless the principle of transgenerational DNA sequence changes now has to be considered a possibility and should be investigated further. At the cellular level, damage leading to chromosome aberrations and mutations persisting through many cell generations has also been observed [5] although not all studies confirm this effect in normal cells (Dugan and Bedford, 2003; Bouffler et al., 2001; Griffin et al., 2000).

Implications for carcinogenesis and genetic risk
What are the implications of untargeted mutations and transgenerational mutation for radiation risk in humans? Risk factors for cancer induction are derived from epidemiology, particularly the effects seen in the A-bomb survivors. The mechanisms by which the carcinogenic changes are brought about are essentially irrelevant to these risk factors except in so far that they might influence the models used for extrapolation to low doses. At present a linear no-threshold model is assumed at low doses and, as stated above, probably the best evidence from actual studies on populations exposed to differing levels of radon is consistent with this model [4]. Nothing that is currently known about repeat sequence mutations seriously threatens this model.

For genetic risk, the situation is perhaps less clear. We know so little about the roles of repeat sequences in vivo. I find it unlikely in evolutionary terms that they are “junk DNA” as some have argued. Certainly a few mutations in such sequences are known to be associated with human disease and they may also affect transcription or mRNA splicing. However, there is no evidence currently that radiation induced changes in DNA repeat sequences are associated with any health effect. One would expect that were such changes to have a heritable health effect they could be regarded for risk factor purposes as being covered by the sorts of models explored by Sankarayaryanan and his colleagues. Of course, if transgenerational mutagenesis were to be shown to be likely in humans then the implications would be different and would need to be considered carefully. Overall, however, there seems no reason to expect a dramatic revision of germline risk estimates in the immediate future.

Gene expression and pregnancy outcome
The only area of possible concern might be pregnancy outcome and the reason is that some repeat sequence changes are known to affect gene expression, in fact the whole area of gene expression and pregnancy outcome following irradiation seems ripe for re-evaluation. Properly ordered differential gene expression is crucial to the proper development of the embryo and foetus. We know from the work of David Barker and his colleagues that dietary restriction associated with low birth weight can lead to a variety of health problems in later life [6]. Changes in gene expression would seem necessarily to be involved in this, and for one case recent work in rats has elucidated the mechanism [7]. A low protein diet initiated at the beginning of pregnancy led to modulation of gene expression in embryonic day 13 metanephroi at a time when nephrons and glomeruli had yet to form. The offspring had fewer renal glomeruli and a higher systemic blood pressure than controls.
That ionising radiation causes multiple changes in gene expression is well known from studies with cellular systems, whole animals (e.g., [8], and humans (e.g., [9] [10] but the doses employed have generally been high. Nevertheless, gene expression can be a sensitive indicator of radiation response and in a myeloid leukaemia cell line linear responses for induction of several genes have been reported down to doses as low as 2 cGy [11]. In primary human fibroblasts more than 200 genes have been reported to be transiently up-regulated by 1 cGy of X-rays [12]. There have also been preliminary reports of changes in gene expression in the descendents of irradiated mice [13] [14] albeit at higher doses.

A few years ago when I was Chairman of the Committee on Medical Aspects of Radiation in the Environment (COMARE) in the UK, I was involved in a review of pregnancy outcome following parental exposure to radiation [15]. We found that almost all the epidemiological studies on pregnancy outcome lacked statistical power due to the low doses to which the populations had been exposed. Only with neural tube defects was there a suggestion (no more) of an association with fathers’ occupational exposure. Epidemiological studies of pregnancy outcome inevitably have concerned themselves with serious effects such as miscarriage, stillbirth, neonatal death or congenital malformation. Any health consequences resulting from changes in gene expression might well be more subtle than these and unlikely to be detectable at or shortly after birth. For example, the changes in gene expression resulting from dietary restriction in humans during pregnancy have been reported to lead in later life to such significant health problems as coronary heart disease, stroke, hypertension, non-insulin-dependent diabetes, and depression [6] [16]. If there are radiation-induced changes in gene expression in the embryo following exposure of parental germ cells or of the embryo itself, the health consequences might well fall within the normal range of variability in the human population and would certainly be difficult to distinguish in epidemiological studies. For ionising radiation this has to be considered in the context of the evolution of human and other life in an environment of measurable background radiation exposure. Even with animals such effects could not be readily studied. Nevertheless, subtle effects which fall within the range of normal variability that exists among otherwise healthy people may still have public health consequences.

However, the technical advances that have become available for the study of gene expression in the last few years have been considerable and for the first time it is now possible to look at changes in gene expression following low dose irradiation of parental germ cells or of the embryo. Such studies would, if negative, reassure us that there is nothing to be concerned about. If positive, however, they could give valuable clues as to the likely health consequences in later life and suggest possible epidemiological approaches.

Acknowledgement

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References