Induced Transgenerational Genetic Effects in Rodents and Humans

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Ionizing radiation/Chromosome aberrations/Delayed mutations.

Delayed appearance of induced mutations has been observed in Drosophila, plants, rodents and recently in humans. The significance of this phenomenon is now recognized especially after the pioneering work of Nomura demonstrating transgenerational tumour induction in mice following treatment with urethane or ionizing radiation. A brief review of the literature on transgenerational genetic effects, namely, chromosomal aberrations and mutations, in rodents and humans is presented here.

INTRODUCTION

The phenomenon of appearance of delayed mutations following treatment with X-rays and chemical mutagens had already been known in the late 1960s in the studies with Drosophila and yeast (for a review, see ref.1 p.270–276). This delayed effect was attributed to the induction of mosaics which become expressed as full mutants in the next generation. Auerbach also realized the possible role of “localized instability” which breaks down repeatedly to generate a mutation as well as “replicating instabilities” throwing out mutant and nonmutant cell lines in the progeny. The possibility of persistence of unrepaired DNA lesions especially those induced by monofunctional alkylating agents which give rise to new mutations in further generations was also considered. It is now recognized that transgenerational genetic effects are mainly due to transmissible induced genetic instability. In this review, transgenerational effects, manifested as chromosomal aberrations and mutations in rodents as well as in humans are considered. Dubrova and Morgan have recently reviewed several aspects of transgenerational effects.

CHROMOSOMAL ABERRATIONS

In vitro studies

It has been shown that cells irradiated in vitro and cultured for several generations are characterized by clonal and nonclonal cells with chromosomal aberrations, the latter arising de novo. Following α-irradiation of murine bone-marrow haematopoietic stem cells in vitro a high frequency of nonclonal chromosome aberrations was observed; this was attributed to the transmission of chromosomal instability from irradiated stem cells to their progeny. Though at that time, it was thought that the observed delayed effect was characteristic of only high LET radiation, later studies showed that such an effect is also observed following low LET radiation. The appearance of nonclonal chromosome aberrations in the progeny of irradiated cells is not unique to haematopoietic cells. A high level of nonclonal chromosomal damage was also observed in human skin fibroblasts exposed to heavy ions and cultured for 25 passages. Radiation-induced chromosomal anomalies progressively decreased during 5 to 7 passages, but showed an increase in later passages and were characterized by aberrations involving telomeric regions of some specific chromosomes. Holmberg and colleagues found delayed clonal and nonclonal chromosomal aberrations in X-irradiated human T lymphocytes cultured in vitro. These studies indicate that several cell types treated in vitro with either low or high LET radiation develop a persistent memory expressed long time after irradiation.

It is of interest to know if the delayed effects observed in vitro is also manifested in vivo. Watson et al. irradiated haematopoietic cells with high or low LET radiation in vitro and transplanted them into radiation–ablated recipient CBA mice and found persistent chromosomal aberrations, demon-
strating that the induced instability \textit{in vitro} can be transmitted \textit{in vivo} as well.

The reverse type of experiments, \textit{i.e.}, in which the cells were irradiated \textit{in vivo} and subsequently cultured \textit{in vitro} have also shown persistent chromosome aberrations for several generations. Weissenborn and Streffer\cite{weissenborn1976} X- or neutron-irradiated one or two cell embryos \textit{in vivo} and studied further cell divisions \textit{in vitro}. They found chromosome-type aberrations and chromatid-type aberrations as well as micronuclei in further cell divisions \textit{in vitro}. This type of experiments was extended by Ullrich and Davis\cite{ullrich1973} who irradiated mice and at different times after radiation, removed the mammary glands which were cultured \textit{in vitro}. They found persistent chromosomal aberrations in these cells.

Both these types of studies demonstrate that cells irradiated \textit{in vitro} or \textit{in vivo} and then allowed to proliferate either \textit{in vivo} or \textit{in vitro} respectively, can continue to generate new chromosomal aberrations.

\textbf{In vivo studies}

Several studies have been undertaken to evaluate the long term effects of \textit{in vivo} irradiation of mice in somatic tissues, with conflicting results. The mouse strain employed and the target cells analysed appear to be important. In CBA/H mice, following irradiation a constant frequency of chromosomal aberrations was observed up to 17 months, which increased to about 50\% at 24 months.\cite{braun1973} No delayed occurrence of chromosome aberrations, measured as micronuclei in erythrocytes was found at 35 days following low dose rate gamma irradiation of mice.\cite{bouffler1973} Bouffler \textit{et al}\cite{bouffler1973} did not find any transmissible chromosomal aberrations in CBA/H mice following \textit{in vivo} exposure to alpha particles or X-rays 50 or 100 days after exposure. In experiments designed to evaluate the persistence of radiation induced translocations and dicentrics, no delayed appearance of aberrations in the peripheral blood lymphocytes, was found in C57BL/6 mice up to 12 months.\cite{braun1973} Similarly, we did not find any delayed appearance of aberrations in either bone marrow cells or spleenocytes of Swiss mice up to 100 to 300 days following X-irradiation.\cite{uma1980}

Uma Devi and coworkers,\cite{uma1980,uma1981} have studied the effects of fetal irradiation and scoring aberrations in adult mice. Following gamma-irradiation of mice on day 14–17 of gestation, a significant dose dependent increase in the frequencies of chromosomal aberrations was found at 12 month of age. In a recent study,\cite{uma1982} they found that pre-treatment of mice to the plant favanoids, orientin or vicenin, reduced the initial frequencies of induced aberrations as well those appearing later (observed up to 12 months) in parallel with the incidence of tumours in various organs.

\textbf{TRANSGENERATIONAL EFFECTS}

\textbf{Chromosomal aberrations}

The trans-generational transmission of damage induced by gamma rays (3 Gy) has been studied by evaluating chromosomal aberrations in the regenerating liver at different times following partial keratectomy as well as in the progeny of irradiated male rats.\cite{shibata1980} In a subsequent study, also with rats, Slovinska \textit{et al}\cite{slovinska1981} extended their observations to proliferating activity of hepatocytes and apoptosis following irradiation and partial hepatectomy. These data showed a reduced proliferating activity, a higher frequency of chromosomal aberrations and a higher proportion of cells with apoptotic DNA fragments, compared with non-irradiated controls. In the progeny of irradiated rats, (F1 and F2 generation) following partial keratectomy, the observed biological effects persisted, but to a lesser degree. The effect of an additional gamma-irradiation (3 Gy) to the progenies was lower than expected on the basis of additivity, probably reflecting an adaptive response.\cite{slovinska1981}

Similar studies concerning the sensitivity of cells derived from the progeny of irradiated parents to a further treatment with radiation or cyclophosphamide were carried out by Vorobtsva.\cite{vorobtsva1981} She had reported earlier an increased sensitivity to X-rays of hepatocytes in the progeny of irradiated rats.\cite{vorobtsva1981} In these studies,\cite{vorobtsva1981} the progenies of X-irradiated rats received 2 Gy of X-rays or cyclophosphamide (25mg/kg body weight). Frequencies of chromosomal aberrations were determined in regenerating hepatocytes (for X-rays) or bone marrow karyocytes (for cyclophosphamide treatment) at different times of recovery. At all time intervals, the frequencies of aberrations in the cells derived from the cyclophosphamide-treated progeny of irradiated parents were significantly higher than in the irradiated controls which received no cyclophosphamide treatment. Similarly, fetal embryonic fibroblasts derived from animals mated with irradiated males, showed an increase in the frequencies of chromosomal aberrations following 3 Gy irradiation, in comparison to those derived from animals with no radiation history. In an earlier paper, Vorobtsova\cite{vorobtsva1989} reported an increased sensitivity to \textit{in vitro} gamma-irradiation of the lymphocytes from children born to patients following anti-tumor radio- and chemotherapy. It is instructive to note that the two studies with rats\cite{vorobtsva1981,vorobtsva1989} mentioned earlier produced contradictory results, one finding an adaptive response\cite{vorobtsva1989} and the other reporting an increased sensitivity of the cells derived from the progeny of irradiated parents.\cite{vorobtsva1989} It is difficult to clarify the reasons for the differences in the obtained results, which may be due to differences in the strains of rats used, the doses of radiation and different protocols employed.

\textbf{Mutations}

\textbf{Mouse:}

The earliest report of transgenerational mutagenesis in mice was by Luning and coworkers\cite{luning1980} in 1976. They detected dominant lethals (early and late embryonic death) following intraperitoneal injection of 239Pu citrate solution in mice. The increase in dominant lethality was found not only in the
Germ line of male mice directly irradiated but also in the F₁ germ line. Lethal and teratogenic effects of X-rays in two successive generations in the HLG mouse strain following irradiation of zygotes were reported by Pils et al.25 These results were confirmed by Hales et al.,26 who treated male rats with cyclophosphamide chronically (for 4 or 18 weeks), mated with untreated females and found significantly increased frequencies of dominant lethals and malformed foetuses in both F₁ and F₂ generations. The occurrence of dominant lethality can be influenced by many factors other than the genetic ones and therefore can provide only indirect evidence for an elevated mutation rate in the offspring of exposed parents.27

Therefore, it is important to look for specific well defined mutations for the persistence of such events in the progeny. The only mutation detection system in germ line of mice, that is widely used is the specific locus method developed by Russell and Russell27 for coat colour genes. However, the mutation rate is very low at these loci and the sensitivity is not high enough to detect transgenerational increases in the mouse germ line.29

A transgenic mouse system carrying a lambda shuttle vector harbouring the lacI gene was used to study the mutation frequencies in the haematopoetic cells of the offspring of irradiated male parents.28 There was a two fold increase in the mutant frequency at 4 Gy indicating a transgenerational transmission of factor(s) leading to genetic instability in the F₁ progeny resulting from preconceptional parental irradiation.

Dubrova and co-workers have developed a sensitive technique to detect mutations in the mouse germ line.29 This technique employs highly unstable expanded simple tandem repeats (ESTR), or unstable minisatellites. These ESTR loci consist of homogeneous arrays of relatively short repeats (4–6 bp) and exhibit a very high spontaneous mutation rate both in germ line and somatic cells.30 The studies of Dubrova and co-workers in mice showed that (a) transgenerational mutations are induced in ESTR loci both by low and high LET radiation, (b) such mutations are induced in all strains of mice tested, (c) there are strain differences in transgenerational effects and (d) mouse strains BALB/c and CBA mice are more radiosensitive than other strains studied.30,31

**Human studies:**

Extensive studies on the progeny of the population exposed to radiation from atom bombs in Hiroshima and Nagasaki did not show any increase in any adverse hereditary effects or cancers. There is also no evidence for increase of any adverse effects attributable to radiation or chemotherapy in children of patients treated for cancer. One of the problems is that the “spontaneous frequencies” of these events are rather high in human populations and therefore it is difficult to detect any significant increase unless a very large population can be investigated.

Dubrova and co-workers have used mutations in minisatellite repeats to investigate as to whether exposure to ionizing radiation can induce transmissible germ cell mutations, employing selected populations, namely the individuals living in the vicinity of the site of the Chernobyl reactor accident (rural areas of the Mogilev district of Belarus, rural areas of Kiev and Zhiomir regions of Ukraine) and those living in the vicinity of nuclear testing site (Semipalatinsk, Kazakhstan) from the former USSR.32–34 In all these studies, they found an increase in minisatellite mutations in the progeny of the exposed parents. In their first study,30,32 the control cohort was from another ethnic origin (from United Kingdom) and hence raised some criticisms. In the study on the Ukraine population, the control and exposed groups were composed of families containing children conceived before and after the Chernobyl accident.33 In subsequent studies, children born to liquidators (clean-up workers of the reactor following accident) also showed an elevated mutation rate in some minisatellite loci.35,36 All these studies clearly demonstrate that there is an increase in the frequency of transmissible mutations following parental exposure to radiation. It should be pointed out that several similar studies have been carried out on the children of exposed parents, mainly “clean-up” workers following Chernobyl accident, with negative or non-significant results. These workers had been, on the average, exposed to about 0.25 Gy as determined by the film badges and subsequent biological dosimetry using persistent chromosome translocations in the peripheral lymphocytes.37 One study showed no elevated rates of mutations in minisatellite repeats, though there was an indication of an increased level of mutations in children conceived when the fathers were working at the reactor site.38 In an interesting study, a comparison was made of the minisatellite mutation rates of 155 children born to Estonian clean up workers after the accident with those of their siblings.39 The mini-satellite mutation rate was non-significantly increased among the children born after the accident with an indication of an increased mutation rate among offspring born to workers who had received doses of 20 cSv or above.39 In another study on the children conceived before and after their father’s exposure during the clean-up duty after Chernobyl accident, no indication of increased mutation rate was found.40 A recent comprehensive study,41 on the children of Chernobyl liquidators by Nomura and co-workers, using 72 microsatellite loci (31 autosomal, one X-linked and 40 Y-linked) did not show any increase over the control children. Mutation rates in hypervariable minisatellite loci in children of atomic bomb survivors (exposed to > 1 Sv) were investigated and no increase was found over the children of unexposed parents.42

It is thus, the human data are controversial. There could be several reasons for this, like type of radiation, doses, age at exposure and other environmental factors. Moreover, the genetic consequences of mutations in mini- or microsatellite loci are not known and these can only be taken as an indi-
icator of transmissible effect of an exposure to radiation or a chemical carcinogen.

REFERENCES

31. Barber, R., Plumb, M. A., Boulton, E., Roux, I., Dubrova, Y.


