

LA-UR 93-0209

CONF-9210291-1

Los Alamos National Laboratory is operated by the University of California for the United States Department of Energy under contract W-7405-ENG-36

LA-UR--93-209

DE93 007379

TITLE RADIATION MUTAGENESIS FROM MOLECULAR AND GENETIC POINTS OF VIEW

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SUBMITTED TO Proceeding for the International Workshop on Radiation Effects
Taipei Taiwan
October 18, 1992

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Radiation Mutagenesis from Molecular and Genetic points of View

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**Proceeding for the International Workshop on Radiation Effects, Taipei,
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Abstract

An important biological effect of ionizing radiation on living organisms is mutation induction. Mutation is also a primary event in the etiology of cancer. The chain events, from induction of DNA damage by ionizing radiation to processing of these damages by the cellular repair/replication machinery, that lead to mutation are not well understood. The development of quantitative methods for measuring mutation-induction, such as the HPRT system, in cultured mammalian cells has provided an estimate of the mutagenic effects of x- and γ -rays as well as of high LET radiation in both rodent and human cells. A major conclusion from these mutagenesis data is that high LET radiation induces mutations more efficiently than g-rays. Molecular analysis of mutations induced by sparsely ionizing radiation have detected major structural alterations at the gene level. Our molecular results based on analysis of human HPRT deficient mutants induced by γ -rays, α -particles and high energy charged particles indicate that higher LET radiation induce more total and large deletion mutations than γ -rays. Utilizing molecular techniques including polymerase chain reaction (PCR), Single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and Direct DNA sequencing, mutational spectra induced by ionizing radiation have been compared in different cell systems. Attempts have also been made to determine the mutagenic potential and the nature of mutation induced by low dose rate γ -rays.

Defective repair, in the form of either a diminished capability for repair or inaccurate repair, can lead to increased risk of heritable mutations from radiation exposure. Therefore, the effects of DNA repair deficiency on the mutation induction in mammalian cells is reviewed.

Introduction

Mutation is a primary event in the etiology of cancer. Mechanistically, point mutations are involved in the activation of oncogenes and deletions are responsible for the inactivation of suppressor genes in many human cancers (9). Cytogenetic events such as translocation and nondisjunction that will affect the expression of multiple genes in a chromosome are also associated with very important classes of human cancer (10). While most of the early work on mutation induction involved the use of animals, recent technical advances in somatic cell culture have allowed populations of human or rodent cells to be used in mutation studies. The inducible recessive and X-chromosome linked genetic marker in mammalian cells, 6-thioguanine resistance, results from the reduction in the activity of the purine salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT). This genetic marker has been widely used in studies of radiation mutagenesis, especially in rodent and normal human fibroblasts (1-8).

RBE for mutation induction

The development of quantitative methods for measuring 6-TG^r mutation induction in cultured mammalian cells has provided an estimate of the mutagenic effects of X- and γ -rays. These methods have also been used to study radiation mutagenesis of higher LET in both cultured rodent and human cells. Higher RBE values for mutation induction than for cell inactivation have been observed after exposure of rodent or human fibroblast cells to accelerated heavy ions (5), fast neutrons (6) and α -particles (7). It has been shown that in mouse and human cells, the RBE for mutation induction at the HPRT locus increase as a function of LET and reach a maximum in the range of 90-200 KeV/ μ m with an RBE value between 6-7 (3-8). Studies by Tsuboi et al., (50) and that by Hei et al., (8) on human skin fibroblasts have shown an increase in RBE as function of LET with a maximum reached at about 150 KeV/ μ m. At higher LET value, the RBE for mutation induction decrease, similar to the RBE for cell killing. The major conclusions of these studies lead us to conclude that irradiation with high LET radiation, such as α -particles, produces more mutagenic events per unit dose in human fibroblasts than does irradiation with X-rays. The large RBE for 6-TG^r mutations can be interpreted to mean that high LET radiation induce more unrepaired and misrepaired DNA damage leading to 6-TG^r mutations than do X-ray at equal survival levels (7).

Molecular analysis of mutation

Classically, the molecular spectrum of the mutants induced by a mutagen is determined by Southern analysis. In this approach, genomic DNA of mutant cells is digested to completion with restriction endonucleases of choice, electrophoresed through agarose gels, and analyzed by Southern blot techniques with hybridized ^{32}P -labeled human HPRT cDNA. When changes are seen, the region of the gene involved is determined by an altered restriction fragment(s). The molecular analysis of mutations at the gene level has been greatly facilitated by the recent development of polymerase chain reaction (PCR)-based DNA amplification. PCR can be used to rapidly identify deletions in genomic DNA by determining whether a specific region of DNA can be amplified. Southern analysis is not only time consuming but may also provide incomplete results due to the limited resolution and the cross hybridization with the pseudogenes. Recently, multiplex PCR technique has been developed which can amplify all 9 exons of the HPRT gene simultaneously in a very short period of time for a large number of samples (51,52).

Molecular genetic analysis of mutations induced by sparsely ionizing radiation at selectable loci of mutants has revealed major structural alteration at the gene level (12-17). Utilizing both Southern and multiplex PCR analyses, a large number of γ -ray, α -particle, argon ion (470 MeV/u)-induced HPRT deficient human mutants have been analyzed. The structural alteration of the HPRT gene of the mutant DNA was compared to the control DNA pattern. Among a total of 109 γ -ray-induced mutants, 30% were total deletion mutants and 24% showed either intragenic or partial deletion. All nine exons were synthesized from the rest of the mutants (46%) without major alteration. On the contrary, ~50% of the α -particle-induced mutants were total deletion and 10% showed a partial deletion of the target gene. Similarly, the percentage of gross gene deletions increases when human fibroblasts are irradiated with higher LET Argon particles. These results indicate higher LET radiation induces more total and large deletion mutations than γ -rays, and it is consistent with the limited α -particle results reported by Thacker et al. (13) using rodent and human cells at the HPRT locus and with data reported by Kronenberg and Little (17) on thymidine Kinase (TK) mutants of human B-lymphoblastoid cells induced by neutrons and ^{40}Ar ions (470 MeV/u). From these data can be interpreted that high LET radiation not only induces mutations more effectively per unit of dose than γ -rays but also induces mutations qualitatively different from those induced by sparsely ionizing radiation.

Molecular mechanisms of radiation mutagenesis

Southern or PCR analysis of radiation-induced mutations can not detect point mutations and small deletions (<50 bases) and also can not tell us the size of those deletions in mutants that extend beyond the boundaries of the target gene. Recent advances in molecular biology enable us to detect mutation from a single nucleotide change to megabase deletions. Utilizing molecular techniques including polymerase chain reaction (PCR), Single-strand conformation polymorphism (SSCP), denaturing gradient gel electrophoresis (DGGE) and direct DNA sequencing, mutational spectrum induced by ionizing radiation have been compared in different cell systems.

The total spectrum and extent of the mutations induced by ionizing radiation in mammalian cells is not clear. In microbes, good genetic evidence suggests base substitutions are the most common type of mutational event caused by ionizing radiation (18-20). Both transitions and transversions have been found at A.T as well as G.C base pairs. These base substitutions appear to be random (18,19). Point mutation analysis in mammalian cells are very limited. Recent studies at the adenosine phosphoribosyltransferase (APRT) locus in CHO cells have also demonstrated that γ -rays can induce frameshifts, transitions, transversions, and small deletions that cannot be detected by Southern blot analysis (21-23). Even though the data base is still small, it appears that base substitutions represent ~75% of the γ -ray-induced point mutations analyzed and the remainder are small deletions. These base substitution mutations are well distributed throughout the APRT coding sequence with every possible type. This wide variety of base substitutions is consistent with the broad specificity of ionizing radiation-induced base substitutions in prokaryotes and in lower eukaryotes. In contrast, the spontaneous mutational spectrum at the APRT locus is characterized by prominent hot spots and multiple independent events at several positions of the APRT gene (21-23). Utilizing the *E. coli* supF gene encoding the suppressor tyrosine tRNA in a human shuttle plasmid as a target, Waters et al. (24) and Jaberaboansari et al. (25) have analyzed a large number of spontaneous, X-ray- and α -particle-induced mutations in human lymphoblastoid cells. Contrary to the APRT system, the distribution of mutations is highly nonrandom in the shuttle plasmids and remarkably similar in both irradiated and control DNA. Since DNA in solution is irradiated before transfecting to the human cells, extensive oxidation effects on the naked DNA may be a contributing factor. Using DGGE technique Okinaka et al. has analyzed γ -ray-induced point mutation in the population of human lymphoblastoid cells (53). The γ -irradiated populations contained several mutations in the low melting region of exon 3 that were not observed in the untreated culture. Two exon 3 specific

mutations were observed in more than one treated culture and various tests for potential biases suggested that these were radiation specific mutational hotspot. These two recurring mutations were specific 1bp deletions in either a run of 4 T:A's or a run of 3A:T's (53). Turkington and Struss (54) have recently suggested that when a damaged thymine is located in a run of T's, a more likely bypass mechanism is through slippage and misalignment which results in the production of one and two bp deletions. They attempt to demonstrate this process in an *in vitro* DNA polymerase assay that "rescues" X-ray damaged plasmid containing a Lac reporter sequence. The similarity between Turkington and Struss data set and the human HPRT exon 3 result suggests that base damage and slippage and misalignment at runs of T's is a plausible mechanism by which ionizing radiation can induce mutational hotspots (53).

Physical characteristics of the different types of radiation should play an essential role on the radiation-induced mutational spectrum. Most importantly, these radiation-specific characteristics will serve as a constant parameter regardless of the physiological conditions of the cells being studied. X-rays and γ -rays generally have a low rate (0.2-3 KeV/ μ m) of liner energy transfer (LET). They penetrate deeply but generate ions sparsely along their path. High concentrations of energy-dissipation events occur within small volumes but per unit dose are less frequent and more widely spaced than high LET radiation, such as α -particles (LET at 100 KeV/ μ m). A direct energy deposition event of low LET radiation will cause DNA strand breaks. The probability that one event will disrupt DNA double strand breaks is small but it will be increased as a function of dose. It has been suggested that low LET radiation exerts the bulk of its damaging effects through oxygen derived free radicals, particularly hydroxyl radicals. Hydroxyl radicals cause modification of the nitrogenous bases, single-and double-strand breaks and base-free (AP) sites (26-28). Recent evidence has suggested that base lesions may be at least equally or twice as common as strand breaks in low LET radiation induced DNA lesions (28,29). An oxidative product has been found in X-irradiated DNA, 8-hydroxyguanosine and it induces transition and transversion mutations involving G.C pairs (30). The action of high LET radiation may differ from that of γ -rays due to the high energy deposition along the ionization path. Free radical-mediated oxidative damages are at least partly contributed in high LET induced DNA lesions. If radiation-induced point mutations are due to the oxidative damages of the DNA mediated by free radicals, it is possible that both low and high LET radiations induce similar types of point mutations. It is also essential to know whether these radiation-induced point mutation spectra are different from that which occur spontaneously.

The basic mechanisms of radiation induced deletion mutations is also not clear. The average energy deposited per absorption event by a low-LET radiation, ~60 eV, is large enough to break any molecular bond. Clusters of ionizations can be produced whose dimensions are large enough to rupture a number of bonds in close proximity due to direct action, or to direct plus indirect action due to the diffusion of reactive species (e.g. OH⁻). For high LET particles, such as α -particles (3.6 MeV), we can imagine the radiation track as a reasonably straight thin cylinder with high energy deposition events along its path. A model has been proposed by Mazumder and Chatterjee (31,35) to calculate the radial energy deposition around a high LET particle track for application in radiobiology. In this model, the structure of a particle track is divided conceptually into two regions: "core" and "penumbra". For a 3.6 MeV α -particle, the core radius of the radiation track, which primarily consists of high energy deposition events, is about 5 Å. The penumbra radius of the track, which is about 9.6×10^2 Å, is a low energy density region (35). More than half of the total energy of the α -particle is deposited in the core region (35). This type of high-LET radiations are very efficient in causing DNA double-strand breaks (DSB) and these types of breaks are either not repaired at all or misrepaired (32-34). It is thought that these types of unrepaired and/or misrepaired DSB could give rise to deletion mutations. We can hypothesize that deletion mutations may occur after cells rejoin either a single DSB or two DSBs followed by recombinogenic mechanisms, chromosomal rearrangement, or by looping out of an interstitial chromosomal segment. It is most likely that a spectrum of deletion mutations can be induced depending on the types of DNA damage and the subsequent repair mechanisms involved. Currently available molecular data suggest that high-LET radiation may induce more and/or larger deletion mutations around the target gene (13,17,36).

DNA repair and mutagenesis

DNA repair plays an essential role in radiation mutagenesis. There is evidence in normal cells that some recovery processes allow mammalian cells to tolerate damage that has not been removed. This action can subsequently result in mutation. Cells that are deficient in the DNA repair process may also restore a damaged chromosome to its original configuration in a misrepaired fashion that may allow cell survival, but may also be mutagenic. The reduction of cell killing and mutation frequency in mammalian cells by low LET irradiation at low-dose-rates, compared to acute irradiation, have been attributed to the action of cellular repair processes during the irradiation (37-41).

This is supported recently from the studies of mutation-induction using DNA double strand breaks (DSB) repair-deficient mutants. CHO mutant XRS-6 and mouse mutants M10, LY-S1, and XL830 cells are hypermutable (42,43,45,46). Mutant XL830 cells also lack a dose rate effect on mutation induction frequency (46). However, Evans et al. (44) have shown that DSB repair-deficient mutant LY-S1 cells are marginally dose rate dependent at the heterozygote TK $^{+/-}$ locus, which presumably allow the recovery of multilocus deletions. Surprisingly, TK $^{-/-}$ mutants recovered from the low-dose-rate irradiation are predominantly multilocus deletions. It is suggested that the decreased DSB repair-capability increases the probability of formation of large deletions (44). Since a significant cell cycle redistribution occurs during the protracted exposure of continuing suspension culture of LY-S1 cells, it may complicate the effects of DNA repair on the frequency and the nature of the mutation-induction. Nevertheless, it is likely that mutations induced by low-dose-rate irradiation in the repair-deficient cells may be qualitatively different than that occurring in repair-proficient cells.

As discussed earlier, mutations induced by acute high dose of X or γ rays have exhibited major structural alterations at the gene level. These events may result from a single ionizing electron track. However, with increasing dose of γ -rays, mutations most likely result as a consequence of two or more electron tracks. In the low dose region, particularly when exposures are protracted low-dose-rate γ -irradiation, the number of tracks will be fewer, and it is possible that the damage due to an individual track may be repaired before damage from additional exposure. It is likely that the ratio of radiation-induced larger deletion to point mutation would decrease such that spectrum of mutation from the low dose exposure would be qualitatively different from those irradiated with high dose radiation. It has been shown that in *Neurospora crassa* the frequency of multilocus deletion, but not point mutation is reduced with a decrease in the dose rate of X radiation (47). Similarly the mutations at specific loci in irradiated mice are often associated with large genetic changes, and these mutations exhibit a dose-rate dependence (48). However, there are no molecular data in mammalian cells to substantiate this hypothesis. Molecular dissection of the mutation spectra induced by low-dose-rate versus acute, high-dose-rate γ -irradiation in normal human cells will provide a comprehensive analysis of the mechanisms of radiation-induced gene and chromosome mutations. Normal human fibroblasts are ideal for this type of experiment since they remain viable, noncycling and contact inhibited during prolonged plateau phase (49). Since more than 90% of the human cells are noncycling, studies utilizing plateau phase human fibroblasts should reflect the *in vivo* situation of the dose-rate effects.

Acknowledgment

We are indebted to Mr. Tracy Hanks and Ms. Debra Adair for technical assistance. This work was supported by the U.S. Department of Energy and National Institute of Health.

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