Induction of Genomic Instability by Low Dose Radiation

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Hypothesis and Approach

The hypothesis driving this research was that the induction of genomic instability can be initiated by complex, poorly repaired DNA damage induced by low doses of ionizing radiation that leads to a mutator phenotype.

The approach that we have taken involved the use of a reporter gene (carried by an unirradiated vector) to directly measure mutations occurring at varied times after exposure to varied doses of irradiation. The irradiated cells were thus transfected with the vector at various times after exposure. The vector carried the GFP gene with an inactivating mutation in its promoter. Activation of the GFP and resultant **fluorescent cells** required a mutation to correct the mismatch in the promoter. The vector also carried an active BFP gene with an active promoter to measure the frequency of transfected cells. Blue and green flourescences were measured by flow cytometry or fluorescent microscopy.

Characteristics of the Biological System

We have utilized a colon cancer cell line (HCT 116) deficient in mismatch repair because of the methylation of the *hMLH1* gene on chromosome 3. The results obtained were compared to those obtained in a matched cell line proficient in mismatch repair via an active chromosome 3 (HCT116 + chromosome 3). It was thought that if genomic instability created errors during DNA polymerization, expression of the GFP protein would occur. The cells were transfected **after** exposure to ^{137}Cs γ radiation.

Measurement

An increase in GFP fluorescence was measured in the interval of 50h to four days following transfection. The number of cells showing GFP was divided by the number of transfected cells as determined by their blue fluorescence emitted by the active BFP protein. The measurement was made during the interval of 50h to four days following transfection in order to correct for mutations occurring during the transfection process. The results were expressed as the percentage of transfected cells expressing GFP per the number of cell divisions occurring during the 50h to 4 day period. The assay was repeated at weekly intervals following exposure.

Results

We observed a linear increase in cells with GFP fluorescence with doses of 0.3 and 1.0 Gy during the first week following transfection (3-5 cell divisions) (Fig; 1A). Although the percentage of delayed mutations was slightly higher for the misimatch repair-proficient cell line than the deficient line (Fig. 1A), this difference between the two cell lines was not apparent in further experiments. When the cells were transfected at later post-irradiation times, the percentage of cells expressing GFP decreased for both cell lines.

Treatment of the cells with accelerated ^{56}Fe ions (0.30 Gy) or hydrogen peroxide (30 μM) caused an increase in the percentage of GFP expressing cells. At higher doses of either agent, the percentage decreased Fig1B, 2). The optimum time period for transfection was 12-14 generations after exposure to ^{56}Fe and 27 generations after treatment with hydrogen peroxide. The increase in the percentage of GFP expressing cells was greater after exposure to ^{56}Fe and hydrogen peroxide than after exposure to ^{137}Cs. The percentage of cells expressing GFP was somewhat greater in the mismatch repair-proficient than in the deficient cell line after treatments with ^{56}Fe or hydrogen peroxide.

Although the results were quite reproducible within a single experiment, we encountered difficulties with reproducibility from experiment to experiment. In order to increase the consistency of the results, various changes in the assay were explored. In the initial experiments described above, the ratio of green fluorescence to blue fluorescence provided a direct measure of mutation in the transfected cells. A problem encountered was that the fluorescent intensity of the BFP is more than 20 times less than the intensity of GFP, and it was not possible to completely compensate for the intensity difference using flow cytometry. Substitution of the red fluorescent protein (RFP) for the BFP was not effective, since the vector was toxic and also exhibited cross over of signal with the GFP in the cytophotometric assays.

The measurement of fluorescence by microscopy was then compared to the flow cytometric method initially utilized. The cells were examined microscopically using a fluorescent microscope outfitted with an automatic stage that facilitates inspection of random microscopic fields At each position surveyed after movement of the microscopic stage, digital photographs are automatically taken under exposure conditions that equalize the signals from the BFP and GFP. However, the inconsistency persisted whether the fluorescence was measured by flow cytometry or florescent microscopy.

Another problem has been the high background originating from the transfection process. In a later series of experiments, the fluorescence obtained at four days post transfection did not exceed the amount obtained at 50 hours.

Summary

The results show that delayed mutations occur in these cell lines after exposure to relatively low doses of low or high LET radiation, as well as after treatment with H_2O_2. The occurrence is both dose and time dependent, often decreasing at higher doses and later times. No marked difference was observed between the response of the MMR-proficient and -deficient strains, perhaps indicating that effects on MMR were not involved in the occurrence of the delayed mutations.

Our experience indicates that this method is not sufficiently robust or consistent to be useful in the assay of the induction of genomic instability by low doses of radiation, at least in these cell lines under our conditions.

Fig. 1A. Delayed Mutant Cells Occurring One Week After Exposure of Cells to ^{137}Cs gamma radiation. The mutants are expressed as the percentage of mutant cells in the transfected population. Cells were treated with Cs gamma radiation, and transfected at various times thereafter with an unirradiated vector carrying an inactive GFP gene and active BFP gene. The percentage of transfected cells showing green fluorescence was determined at various times after transfection. The maximum frequency of the delayed mutations occurred 3-5 generations after the irradiation.

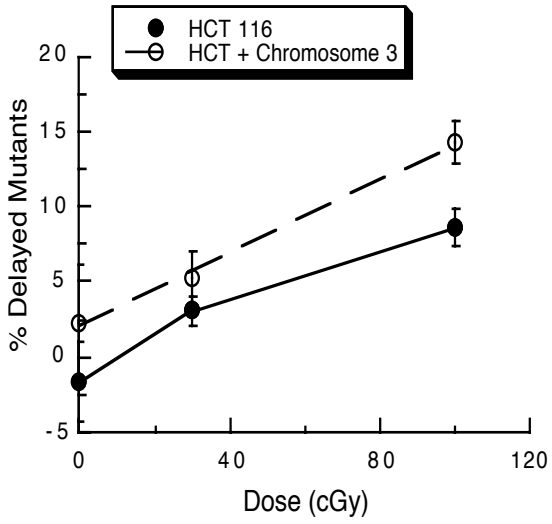
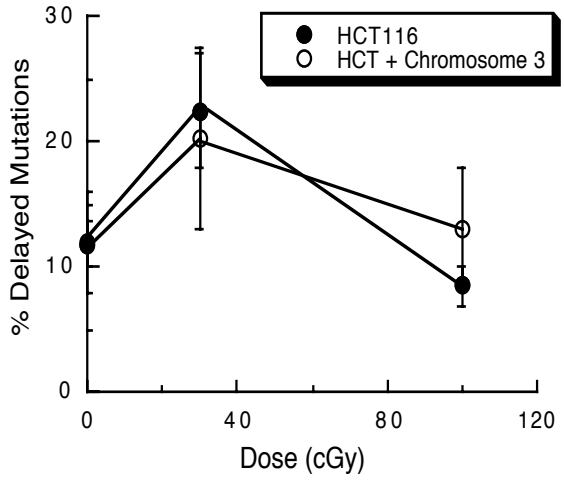


Fig. 1B. Delayed Mutants Occurring Two Weeks after exposure to ^{56}Fe Ions



F**ig. 2. Delayed Mutants Occurring Two Weeks After Treatment with H_2O_2.** A one-hour treatment of the cells at 37° with 30 υM H_2O_2 also resulted in the occurrence of delayed mutants two weeks after treatment. The percentage of mutants was similar to that observed upon treatment with ^{56}Fe ions, and the percentage decreased when measured three weeks after treatment, as seen for ^{56}Fe in Fig. 1B. Slightly more mutants were observed with the MMR-proficient cell line, than for the MMR-deficient HCT-116 cell line.

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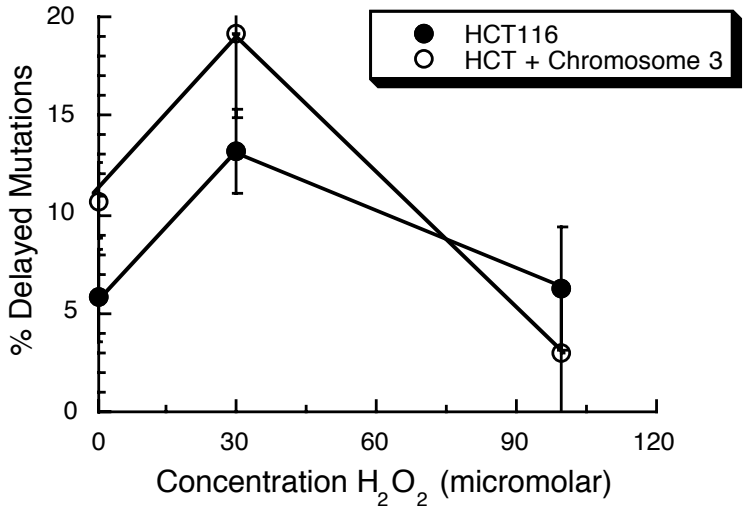
HCT116
HCT + Chromosome 3
% Delayed Mutations
20
15
10
5
0
0
30
60
90
120
Concentration H_2O_2 (micromolar)