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DNA repair and Radiation Sensitivity in Mammalian Cells

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Abstract

Ionizing radiation induces various types of damage in mammalian cells including DNA single-strand breaks, DNA double-strand breaks (DSB), DNA-protein cross links, and altered DNA bases. Although human cells can repair many of these lesions there is little detailed knowledge of the nature of the genes and the encoded enzymes that control these repair processes. We report here on the cellular and genetic analyses of DNA double-strand break repair deficient mammalian cells. It has been well established that the DNA double-strand break is one of the major lesions induced by ionizing radiation. Utilizing rodent repair-deficient mutant, we have shown that the gene(s) responsible for DNA double-strand break repair is also responsible for the cellular expression of radiation sensitivity. The molecular genetic analysis of DSB repair in rodent/human hybrid cells indicate that at least 6 different genes in mammalian cells are responsible for the repair of radiation-induced DNA double-strand breaks. Mapping and the prospect of cloning of human radiation repair genes are reviewed.

There is evidence that DNA repair capacities differ among individuals, and there are well known instances of inherited susceptibility to cancer. Our understanding of the genetics of mammalian DNA repair suggest that a highly complex system is involved. Therefore, the likelihood that there are a number of alleles in the human population that affect susceptibility to radiation effects when present in the heterozygous state must be taken very seriously. Understanding the molecular and genetic basis of radiation sensitivity and DNA repair in man will provide a rational foundation to predict the individual risk associated with radiation exposure and to prevent radiation-induced genetic damage in the human population.

Introduction

A number of laboratories have investigated the mechanisms by which cell killing, mutation, and neoplastic transformation are induced in rodent or human cells in vitro following exposure to UV light, X-irradiation and a variety of chemical carcinogens. When such cells are allowed to repair DNA prior to cell division, repair of DNA damage has been linked to the biological recovery of cells under these conditions (1-3). These findings support the hypothesis that the response of eukaryotes to DNA damaging agents is determined by the effectiveness of a variety of DNA repair systems. Therefore, understanding the nature of the repair and misrepair processes is central to the elucidation of the mechanisms through which subsequent adverse health effects such as mutations, fetal malformations, and cancers are expressed.

Ionizing radiation produces a variety of damage in DNA including DNA-protein cross-links, DNA single strand breaks (SSB), DNA double strand breaks (DSB), and base damage. In prokaryotes and lower eukaryotes, a number of genes involved in the repair of DNA damage caused by ionizing radiation have been identified. In some cases these genes have been cloned and their role in different repair pathways partially characterized (4). These studies have been considerably helped by the use of mutant strains. However, DNA repair mechanisms in mammalian cells are less well understood, primarily due to the lack of suitable mutant cell lines. However, similar approaches to those used in prokaryotes and lower eukaryotes are now possible in mammalian cells with the advent of recombinant DNA techniques and the isolation of a number of mammalian cell mutants that are defective in DNA repair.

In this report we will review recent studies on the genetics of DNA repair in mammalian cells using mutants that are deficient in the repair of radiation-induced DNA double-strand breaks.

Human genetic syndromes

Mutants of microorganisms sensitive to various DNA damaging agents have been of immense importance in helping to elucidate the major pathways of DNA repair in bacteria. In yeast, more than 50 mutants have been isolated that express sensitivity to UV, X-rays or chemical carcinogens and are shown to be deficient in certain repair genes. In humans, individuals afflicted by certain autosomal recessive syndromes are at high risk for cancer. Cells from these patients exhibit both chromosome instability and increased sensitivity to specific DNA damaging agents in culture. Cleaver (5,6) has provided evidence that fibroblasts from

xeroderma pigmentosum (XP) patients are defective in their ability to repair specific lesions formed in DNA as a result of exposure to UV light. Increased sensitivity to X-Irradiation of cultured fibroblasts has been found in a number of autosomal recessive diseases, such as: ataxia telangiectasia (AT) (7), Fanconi's anemia (FA) (8), Cockayne's syndrome (CS) (9), Gardner syndrome (GS)(9), and Rothmund Thomson syndrome (RTS) (10). These genetically disordered patients are apparently deficient in some form of DNA repair. Smith and Paterson (10) have suggested that the primary defect of RTS may be an aberrant gene control function which results in a misdirected repression of a DNA repair gene(s). Several of the XP and CS genes have been identified recently (11-13). However, most of these genes or gene products that are affected in all of these cancer prone disorders are unknown.

Genetic analysis of radiation-sensitive mutants

As an approach to the elucidation and the understanding of mammalian DNA repair, a number of Chinese hamster ovary (CHO) cell lines that are sensitive to UV radiation or chemical carcinogens have been isolated (14-16). A number of ionizing radiation sensitive rodent mutants have also been isolated (14-19). Thirteen of these mutants have been shown to be deficient in rejoining of radiation-induced DNA DSB based on results obtained by neutral elution analysis or pulsed field gel electrophoresis (20-23). Genetic complementation analysis of different mutant cell lines with the same phenotype is often used to determine the number of genetic loci involved in the expression of a particular trait. As indicated in Table 1, a total of13 rodent radiation sensitive mutants have been identified as deficient in DNA DSB repair. Mutants within the same category belong to the same complementation group. Most of the repair deficient mutant cell lines are complemented by normal human fibroblasts or lymphocytes. The first four groups of mutants belong to different complementation groups. All the mouse DSB repair-deficient mouse mutants belong to different complementation groups. Therefore, up to 10 human genes are responsible for the repair of ionizing radiation-induced DNA DSBs.

Cellular repair capability of DSB repair mutant cells

One of the DSB rejoining deficient mutants, XRS-6, has been characterized extensively. XRS-6 cells are highly sensitive to X-rays in terms of cell killing (19), chromosome aberrations (24,25), enhanced mutation induction (26,27), G2 block (28), and the enhanced radiosensitivity of DNA synthesis (24). Our results also indicate that XRS-

6 cells have no potential lethal damage repair (30), no sublethal damage repair (31), and no dose-rate effects (4). Biochemical analyses have also indicated that the ligase (32), topoisomerase (33), and AP endonuclease activities (unpublished results) of XRS-6 cells are normal and the only biochemical defect has been the inability to rejoin DNA DSBs (22,23). A summary of these studies is presented here.

	cell line	Origin	Complementation by human cells	Designed human gene	References
1	XR-1 M10	CHO L5178Y	yes	XRCC4	23, 18
2	XRS-6 XRV15B	CHO V79	yes	XRCC5	29, 16
3	V3	CHO	yes		62
4	XR-V9B	V79	yes		63
5	BLM-2	CHO	yes		64
6	SL3-1478	Mouse L	yes		65
7	LX830	L5178Y	in progress		14
8	L5178Y/S L5178Y/SS	L5178Y L5178Y	in progress		21, 66
9	SX9	Moue SR-1	in progress		67
10	SX10	Mouse SR-1	in progress		61

Table 1. Rodent DNA double-strand break repair-deficient mutants

Repair of radiation-induced DNA SSB and DSB

The yield of DNA SSB in CHO-K1 and XRS-6 cells upon exposure to increasing radiation dose was found to be the same (data not shown). Closure of DNA SSB proceeded at a similar rate in both CHO-K1 and XRS-6 cells with an initial SSB closure half-time of 3 min at 37°C (unpublished results). Induction of DNA DSB was estimated in these two cell lines by pulsed-field gel electrophoresis. Exposure to increasing radiation doses caused an increasing fraction of cell DNA to enter the agarose gel in both CHO-K1 and xrs-6 cells (data not shown). It has been assumed that an

increase in the fraction of DNA radioactivity entering the agarose gel reflected a decrease in the length of nuclear DNA and thus an increase in the frequency of DNA DSB. Rejoining of DNA DSB has been estimated by comparing the amount of DNA in the gel and the plug. After a dose of 50 Gy and a repair time of 4 h the xrs-6 cells showed a qualitatively reduced ability to rejoin breaks measured by PFGE compare to the parental cells. In parental, CHO-0K1 cells the radiation-induced dsbs were rapidly removed. However, the DNA breaks were closed in xrs-6 cells somewhat more slowly, with 50% of the breaks being closed in CHO-K1 and xrs-6 cells after 5 and 12 min respectively (33).

Effect of delayed plating in synchronized G1 cells

Both of the parental and mutant cells were synchronized in G1 by isoleucine depletion (34). These cultures were used for the delayed-plating PLD repair determination and for split-dose recovery in G1 cells. CHO-K1 and XRS-6 cells were inoculated into T-25 flasks and synchronized by isoleucine depletion. Estimated by autoradiographic analysis of cultures with a 60-min pulse of [^{3}H]thymidine (10mCi/ml at a specific activity of 50 Ci/mmol), it was shown that between 10 and 12% of the cells were in S phase. The survival fraction of G1 phase-arrested CHO-K1 cells increased during postirradiation incubation in IL$^-$ medium. As described by Nagasawa et al., (), CHO-K1 cells displayed the typical pattern of PLD repair that saturated about 8 h postirradiation incubation. When PLD repair was measured at two or three different survival levels the recovery ratio increased proportionately with dose. However, XRS-6 mutant cells showed no PLD repair during the postirradiation holding period in IL$^-$medium. Lack of PLD repair has also been observed in other DSB repair-deficient mutants.

Effects of plit-dose irradiation in synchronized G1 cells

We measured the split-dose recovery in parental and mutant cells by delivering two equal dose fractions at various time intervals to cells synchronized by isoleucine depletion (30). There is a large increase in survival for CHO-K1 cells as a function of the time between doses. The repair half-time for the parental cell line was about 90 min and an 4 - 5 fold increase in survival was seen when the doses were separated by 4 -12 hours. By contrast, there is no increase in survival for the sensitive mutant, XRS-6 cells during the interval of dose fraction.

Effects of low dose rate

Dose rate effects were measured in synchronized G1 cells. CHO K1 cells exhibited a large dose rate effect when survival curve generate from exposure to g-rays at 75 cGy/min and 15.3 cGy/h were compared (30). On the other hand, there was no apparent dose-rate effect for the X-ray-sensitive XRS-6 mutant cells, even when the cells were exposed to g-ray at the very low dose rate of 2.7 cGy/h (30).

Radiation mutagenesis

It has been suggested that radiation-induced deletion mutation may result from DNA double-strand breaks since experiments show that both chromosomal aberrations and mutations can be induced by treating cells with restriction endonucleases (35). Evans et al. (36) suggested that the repair deficiency could be responsible for the occurrence of residual DNA multilocus lesions following X-irradiation in the radiosensitive strain LY-S of the mouse lymphoma cell line (L5178Y) which results in its increased mutational response. In addition to mouse LY-S cells, enhanced radiation-induced mutation-induction (hypermutability) observed in several other DSB repair deficient mutants (37,38) suggests that the mutational events outnumber the lethal events induced by DSBs. A plot of mutation frequency versus survival have been analyzed in mutants M10 and XRS-6 (37,39). It is reasonably fitted by a common line suggesting a common lesion type leading to expressed mutations and cell killing in both these cell lines. These results suggest the likely involvement of DSB in mutation induction as well as cell killing in irradiated mammalian cells. It also seems likely that the high mutability of the DSB repair-deficient mutants result from its inability to remove dsb from the DNA resulting in high levels of deletions (40,41).

Cloning of human DSB repair genes

Present attempts to clone human DNA-repair genes rely on schemes which utilize human genomic DNA to complement rodent lines with defined deficiencies in DNA repair mechanisms. Once a complementing human gene can be identified within a deficient rodent background (introduced by DNA transfection), recombinant DNA technologies can be utilized to isolate and clone the human gene. In this approach, DNA from normal human cells is transfected into mutant hamster cells using the calcium phosphate precipitation method. Selective pressure is then applied to the transfected population by treatment with an appropriate agent (e.g., UV, X-rays, chemicals) under conditions that allow only transfected cells that have a normal or wild-type phenotype to survive. It is presumed that

resistant rodent clones have acquired the repair gene of interest by integration of the foreign human DNA. The repair-proficient transformants are often screened with human repetitive sequences to verify the presence of DNA of human origin within the transformants. The human Alu-family sequences constitute a built-in physical genetic marker that allow for the detection and localization of human DNA sequences in transformed hamster cells. In the past 10 years, 6 human DNA repair genes have been isolated. Westerveld et al., (42) Weber et al.,(43), Mudgett et al., (44) and Weeda et al, (45) have used similar strategies to clone 4 human repair genes utilizing CHO mutants deficient in DNA excision repair (ERCCl, ERCC2, ERCC3, ERCC5, ERCC6). A human repair gene responsible for the rejoining of DNA single strand breaks (XRCC 1) has also been cloned by Thompson et al. (46).

There are several major reasons for such slow progress in isolating human radiation DNA repair genes: 1) Current methods for cloning are dependent on functional complementation rather than use of molecular probes; 2) Mammalian cells in general are inefficient at taking up large DNA molecules. In the case of DSB repair-deficient mutants, such as XRS-6, the transfection frequency by cosmids is at least 10-50 fold less than normal CHO cells (unpublished results); 3) DSB repair-deficient mutants are sensitive to ionizing radiation, but radiation itself is not an ideal selection agent for the isolation of repair-proficient transformants.

To avoid all the possible phenotypic drawbacks of the repair-deficient mutants described above, advanced somatic and molecular genetic technologies have been suggested for the cloning of human DSB repair genes. It is well established that functional complementation can be achieved by somatic cell hybridization at a very high efficiency. The human chromosome that complements defective rodent repair phenotypes can be identified from the resulting hybrids between normal human cells and rodent mutants (477-49). Recent development of a high efficiency (>1x10-5) chromosome transfer technique provides an approach for construction of hybrids containing only a single specific human chromosome (50-51). If the radiation-sensitivity of rodent repair-deficient mutant cells can be complemented by a single human chromosome, it means that a repair gene(s) is located on this specific human chromosome. Somatic cell genetic techniques have also been developed to reduce the amount of human chromosomes present in a hybrid cell (52) Therefore, it is now possible to construct a human/mutant hybrid, which contains only a fragment of a specific human chromosome that can functionally complement the mutant cells. Subsequently, unique human sequences or transcripts located on a fragment of a human chromosome will be amplified and isolated by PCR technique using human specific Alu as the primer (53). These isolated human probes from a

known region of a specific human chromosome will then be used to clone the repair gene(s) in YACs which can accommodate large sizes of DNA (100-800 kb).

Mapping of human DSB repair genes

Identification of human chromosomes carrying genes that complement DNA repair deficiencies in rodent mutant cells has been done by somatic cell genetic techniques. Human cells were fused to rodent mutant cells and hybrids were selected at levels of DNA-damaging agent at which the mutant cells were hypersensitive. Concordance of hybrid clone resistance to the selecting agent with a particular human chromosome (or part of a human chromosome) identifies the location of the complementing gene -- Excision Repair Cross Complementing (ERCC) or X-ray Repair Cross Complementing (XRCC). Such studies have mapped ERCC1, ERCC2 and XRCC1 onto human chromosome 19q13.1-q13.3 (54-56), ERCC3 and ERCC5 onto chromosome 2 and 13, respectively (49), and XRCC4 onto chromosome 5 (50). Furthermore, similar preliminary data suggest the location of ERCC4 on chromosome 16p13.13-p13.3 (58), and that of XRCC2 and XRCC3 on chromosomes 7 and 14, respectively (59).

Radiation resistance or sensitivity of somatic cell hybrids constructed from the fusion of XRS-6 cells with primary human fibroblasts strongly correlated with the retention of human chromosome 2 isozyme and molecular markers. Disconcordancies between some chromosome 2 markers and the radiation resistance phenotype in some of the hybrid cells suggested the location of X-ray Repair Cross Complementing 5 (XRCC5) gene on the chromosome 2 (60). Introduction of human chromosome 2 by microcell-mediated chromosome transfer into the radiation sensitive XRS-6 cells resulted in hybrid cells in which the radiation sensitivity was complemented (39, 60).

DNA repair and radiation protection

There is evidence that DNA repair capacities differ among individuals, and there are well known instances of inherited susceptibility to cancer. Our understanding of the genetics of mammalian DNA repair suggest the involvement of a highly complex system. It also clear that certain DNA-repair-related genetic disorders are associated with autosomal recessive diseases, such as XP and AT. Even though human genetic disorders related to DNA DSB repair have not been identified yet, it might be expected the heterozygotic state of some of these DSB repair genes or these disease-associated alleles would have some phenotypic effect. This could have

quite an important effect on the population. Nagasawa et al., have measured radiation sensitivity among ten apparent normal human diploid fibroblast strains based on three different end points (26). They have found that three of these ten strains showed a moderate moderate degree of hypersensitivity to X-rays by all three assays, falling within the range previously reported for AT heterozygotes (27). Therefore, the likelihood that there are a number of alleles in the human population that affect susceptibility to radiation effects when present in the heterozygous state must be taken very seriously. Understanding the molecular and genetic basis of radiation sensitivity and DNA repair in man will provide a rational foundation to predict the individual risk associated with radiation exposure and to prevent radiation-induced genetic damage in the human population.

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