



Low Dose Radiation Increases DNA Repair

Lye Meng Markillie and Eric J. Ackerman

Cell Biology and Biochemistry Group, Biological Sciences Division,
Pacific Northwest National Laboratory, Richland, WA



Abstract

We developed a **functional** assay to measure the effects of LDR on repair of many different lesions representative of those found in cells as consequences of normal oxidative metabolism, as well as those caused by radiation. Currently only 1/10th attomole = 10^9 damaged molecules/cell and 3000 cells/measurement are required. We have found that even low doses (10 rad) exert measurable effects on DNA repair. Interestingly, the amount of DNA repair **increases** at 10-50 rads, plateaus, and then increases even further at higher doses well below doses where radiation-induced lethality would be expected, and surprisingly repair increases even at doses causing decreased cell survival. Our X-RAD 320 Pantak Seifert irradiator is unreliable below 5-10 rads, so we do not know if even lower doses might also stimulate repair. Thus, the sensitivity of our assay exceeds the capability of our irradiator to generate sufficiently reliable low doses to determine the lower boundary for stimulation of repair by LDR.

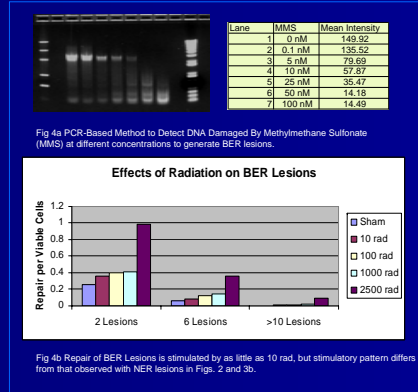
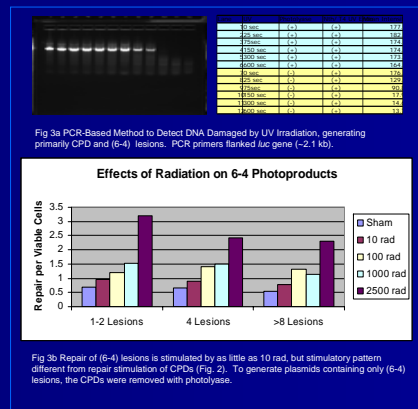
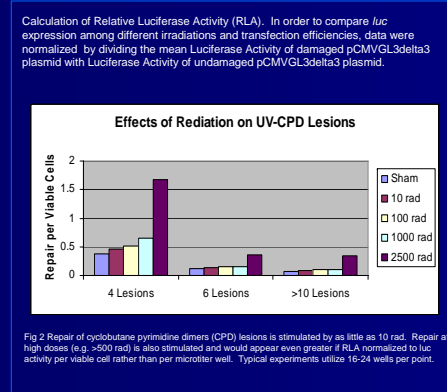
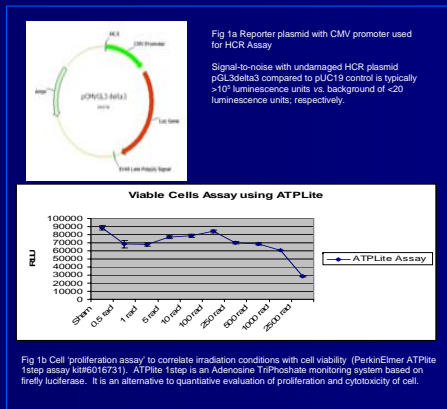
We are not measuring the damage to chromosomal DNA, if any, caused by LDR. Our assay is host-cell reactivation (HCR), so a selected type of damage to DNA is placed in a reporter gene; *i.e.* *luc*. If the damaged is not removed, then there is no luminescence. If damage is removed, luminescence is detected and quantitated. DNA lesions tested so far comprise those expected to be found as a consequence of normal oxidative metabolism or UV-irradiation. Most of the repair we measured so far is from damage attributed either solely to one of two pathways: nucleotide excision repair (NER) or base excision repair (BER). We determined that low dose differentially affects different NER lesions resulting from UV irradiation, and we also are examining the first-known oxidative lesion repaired only by nucleotide excision repair found in normal cells; *i.e.* cyclo-dA. This lesion is found in normal cells and thought to be a byproduct of oxidative metabolism. Varying parameters such as transection protocols or DNA quantities do not appreciably changed the overall significant result that repair is stimulated at 10-50 rads, followed by a plateau, and then followed by another stimulation near 1000 rads. It is likely that more than one pathway is responsible for these differential effects of LDR on repair. The human fibroblast lines GM00639 and GM04429 have been primarily used to date and the control line incapable of NER behaved as expected with NER lesions; thus, the HCR assay reflects identical diminished NER found in human patients. We have begun additional experiments to gain mechanistic insights into the above-noted findings. In particular, we have employed reagents that affect transcription to determine whether repair stimulation is global, or an example of transcription-coupled repair. Demonstration that repair is stimulated by LDR should open a new area of investigation and it has many implications for human health. For example, do some individuals exhibit a greater, lower, or no stimulation to certain lesions? DNA repair and DNA metabolism utilize hundreds of genes, so polymorphisms in these genes could reflect differential effects of LDR on repair of particular lesions. Thus, it would be possible to use our assay to screen individuals for enhanced/decreased ability to repair certain lesions +/- LDR.

Introduction

The vast majority of DNA damage is produced by essential metabolic processes generating free radicals and oxidative by-products, rather than by natural (e.g. sunlight) and man-made (e.g. radiation) sources. Since LDR is unlikely to cause many additional lesions, if any, the purpose of our project is to measure the impact of LDR on DNA repair of the hundreds of thousands of endogenous lesions that occur per cell per day. The ability to repair DNA damage constitutes a vital requirement in human pathology. A single unrepaired, or an incorrectly repaired lesion, dwarfs the consequences of thousands of correctly repaired lesions because unrepaired or incorrectly repaired lesions lead to mutations, and mutations coupled with promotion (proliferation) cause cancer. An accumulative lifetime of misrepaired lesions may even explain known measurable declines in repair and fidelity, the multi-step nature of cancer development, and the aging process. Defects in DNA repair lead to defective embryogenesis, tissue-specific dysfunction, hypersensitivity to DNA damaging agents, premature senescence, genetic instability, neurological and elevated cancer rates. Polymorphisms in DNA repair or repair responses to stressors such as LDR could lead to more subtle effects that accumulate over a lifetime to yield cancers or neurological effects. A sensitive, automated, quantitative method for testing DNA repair against a library of lesions will be an important tool in evaluating health risks arising from low dose radiation.

Materials & Methods

HCR Assay: A reporter plasmid pGL3delta3 construct containing the luciferase gene was either irradiated using a 254-nanometer ultraviolet light lamp (to generate CPD or (6-4) lesions [4]) or treated with chemical MMS (to generate BER lesions). Fibroblast cell lines GM00639 and GM 04429 (Coriell Institute for Medical Research, Camden, NJ) were transiently transfected with damaged or undamaged plasmid using Lipofectamine 2000 (Invitrogen, Carlsbad, CA), cultivate for 24 hours for repair and luciferase expression. Cells were lysed using Promega Passive Lysis buffer and luciferase activity was measured using VictorLight counter (Perkin Elmer). Repair is proportional to measured light units.



Conclusions

- Host Cell Reactivation Assay is sufficiently sensitive to detect increased repair of both NER and BER lesions from as little as 10 to as much as 2500 rad. (lowest & highest tested doses so far).
- Different DNA lesions exhibit different patterns for increased repair following radiation.
- DNA repair of lesions not caused by LDR is stimulated by LDR.
- DNA repair of lesions not caused by higher doses of radiation is greatly enhanced, especially if plotted as repair per viable cell.

Future Directions

1. Continue to expand library of substrate DNA's containing various site-specific lesions that represent damage arising from both LDR and endogenous damage.
2. Confirm results in additional cell lines.
3. Determine the effects on DNA repair of low doses of high-LET, HZE particle radiation measured by the HCR assay.

References

1. J. H. Robbins et al, "DNA repair in human fibroblasts, as reflected by host-cell reactivation of a transfected UV-irradiated luciferase gene, is not related to donor age," Mutation Research, 554(1-2):8-17, 2004
2. J.H. Robbins et al, "Xeroderma pigmentosum: an inherited disease with sun sensitivity, multiple cutaneous neoplasms and abnormal DNA repair," Annals of Internal Medicine, 80:221-48, 1974
3. P. J. Brooks et al, "The Oxidative DNA Lesion 8.5'-(8)-Cyclo-2'-deoxyadenosine is repaired by the nucleotide excision repair pathway and blocks gene expression in mammalian Cells," Journal of Biological Chemistry, 278:22355-62, 2000
4. E. J. Ackerman et al, "DNA Polymerase α and β are required for DNA repair in an efficient nuclear extract from Xenopus Oocytes," 271(3):13816-20, 1996
5. J.A. Hartley et al, "PCR-Based methods for detecting DNA damage and its repair at the sub-gene and single nucleotide levels in cells," Molecular Biotechnology, 20:181-96, 2002