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Residual DNA Damage: What is Left Over and How Does This Determine Cell Fate?

Trevor J. McMillan

Radiation-induced DNA damage may remain unrepaired for a number of reasons: it may be too severe, it may be in inaccessible parts of the genome, it may be induced at critical points in the cell cycle or it may be converted into large DNA deletions. This residual damage is likely to be responsible for cell death either by physical restriction of replication or transcription or by metabolic disruption due to loss of function of critical genes. Although residual damage is important, cells may differ in their ability to tolerate it, which may be a factor that determines the relative radiosensitivity of a given cell population.
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INTRODUCTION

IF FULLY efficient, the repair of DNA damage after insult by chemical and physical agents would result in a cell having no remaining damage. With drugs this can sometimes be achieved if the cells are kept in a strictly non-proliferative state, such that within the sensitivity of the appropriate assays, damage is totally removed and cell kill is zero. With ionising radiation the situation is different since there always appears to be a degree of residual damage no matter what the radiation quality, dose, dose-rate or culture conditions.

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At the level of cell survival this is demonstrated by experiments on plateau-phase cells irradiated at low dose-rate in which a terminal slope to the survival curve is reached as the dose rate is lowered (Fig. 1). Because of problems with proliferation such studies are rare but in the one study where this has been done thoroughly [1] this pattern was produced exactly, indicating that there is an amount of nonrecoverable damage produced following treatment with ionising radiation. At the level of naked DNA isolated form irradiated cells the demonstration of residual damage (usually strand breaks) is hampered by the lack of sensitivity of the techniques available. However, rarely has complete rejoining of strand breaks been observed. This is especially true for DNA double strand breaks (dsb) which are believed to be the most significant lesions in terms of cell death.

It is likely that this level of residual damage is important in the determination of the degree of killing by radiation treatment.

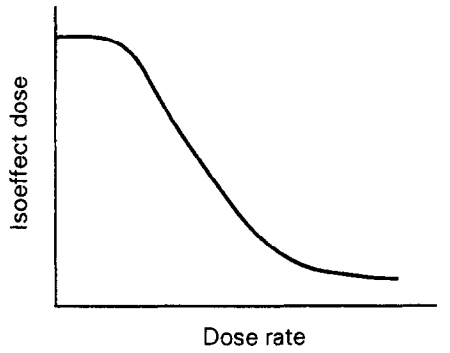

Fig. 1. Dose-rate effect for radiation treatment. As the dose-rate is lowered within a given range the dose for a given level of cell kill is increased. Below this range the isoeffect dose is not altered, suggesting a non-recoverable component of radiation-induced damage.

In this brief article, it will therefore be considered why all radiation-induced damage is not repaired, the molecular consequences of this and how it may determine the toxic effects of radiation.

Determinants of the existence of residual DNA damage

Four possible factors might lead to the persistence of some lesions.

Lesion type. A wide range of types of damage is inflicted in DNA by ionising radiation, including base damage, intermolecular cross-links and breaks in the DNA strands [2]. The repairability of these lesions differ so the residual damage may be the survival of a subset of lesions which cannot be repaired. The nature of such severe lesions may lie in the chemical nature of a single lesion or in the occurrence of several lesions within a short stretch of DNA. The existence of several different chemical termini within a double-strand break (dsb) [3] suggests the first of these. Also it has been demonstrated that restriction endonucleases when introduced into permeabilised cells have different biological effects depending on the nature of the dsb introduced [4]. Blunt ended breaks (those with no overlap) are more efficient at producing chromosomal aberations and cell death than those with an overhang (cohesive-ended). The pattern of energy deposition predicted by computer modelling of the passage of a photon through water is such that energy is deposited in spurs consisting of several ionising events. These spurs have a larger diameter than the width of the DNA strand thus there may be a number of lesions within a short stretch of DNA [2]. These local multiply damaged sites (LMDS) have been suggested to be lesions which are difficult to repair.

Position of damage. There is some evidence to demonstrate that the position of damage in the genome affects its fate. It has been suggested that different regions of the genome may differ in their repairability. This is well characterised following ultraviolet light treatment where it has been found that repair is greater in transcribing than in non-transcribing regions of DNA and indeed in the transcribed rather than the non-transcribed strand of DNA [5, 6]. There is some evidence that this might also be true following ionising radiation [7].

Repair versus fixation. In 1965 Alexander *et al.* [8] published their "competing processes hypothesis" (Fig. 2) in which the fate of damaged DNA was determined by the competition between repair and fixation. One possible explanation of what fixation might be is that a lesion can be transformed into a much larger or different type of lesion before it can be repaired. This modification of damage may in fact be a consequence of the repair processes themselves, i.e. they may be a result of "misrepair". In studies of radiation-induced mutants [9, 10] it was observed that gene markers that were significant distances apart on a given chromosome were lost together. Similarly Powell and McMillan [11] have found large DNA sequence loss in foreign DNA originally containing only single dsb when introduced into cells. These suggest that quite simple lesions (e.g. restriction enzyme-produced, "cohesive-ended" dsb) can be converted into large losses possibly as a consequence of endonuclease activity, by the process of recombination or, in the case of the radiation-induced mutants, by the interaction of two independent lesions.

Phase of cell cycle. Irradiation at different phases of the cell cycle in proliferating cells may have quite different consequences. Apart from differences in induction and repair of damage it has been suggested that within the cell cycle there are certain points at which damage is expressed, i.e. fixation points [12]. So if irradiated at a particular stage of the cell cycle, i.e. just before an expression point, a cell may suffer toxic effects before the damage can be repaired.

Sub-cellular manifestations of DNA damage

The consequences of residual DNA damage can be seen at both the physical and biochemical level. As mentioned above, DNA sequence analysis of specific genes in irradiated cells has revealed large deletions. It is not clear how these arise but they can be reflected in gross chromosomal alterations in cells. At a simple level these can be visualised in interphase cells by inducing premature chromosomal condensation by fusion with a mitotic cell or in post-mitotic cells by the presence of micronuclei. Micronuclei are formed when a chromosome fragment becomes separated from its parent chromosome and forms its own nuclear membrane following mitosis. From these techniques it is difficult to evaluate how these fragments are formed although there is some evidence to suggest that the fragments can contain centromeres (as detected using anti-kinetochore antibodies) so they are not simply the loss of the ends of chromosome arms [13].

Cytogenetic analysis allows a more rigorous analysis of the events which occur after irradiation. A variety of aberrations can be observed [14] but in general it has been concluded that only those which lead to loss of genetic material (e.g. interstitial deletions, dicentric chromosomes) are of significance to cell survival.

At the biochemical level it is evident from the study of non-essential gene loci that radiation can leave behind significant biochemical deficits. For technical reasons these have been largely characterised by the analysis of cells that have been made

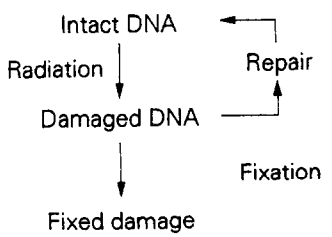

Fig. 2. The competing process hypothesis of Alexander *et al.* [8].

resistant to cytotoxic agents. These have generally been found to be due to large scale changes in DNA although single base-pair changes have been detected in at least one locus [15]. As well as leading to cell death (see below) these biochemical changes can lead to viable, but phenotypically modified cells. Alterations in malignancy, either in terms of the initial carcinogenic event or in the advancement of tumour progression, are of significance. Also radiation-induced alterations in sensitivity to cytotoxic drugs has been demonstrated in human tumour cells [16].

How does residual damage lead to cell death?

There are two possible answers to this, a physical one and a biochemical one. Physically the existence of damage may halt initiation of replication or transcription or halt the progression of these processes. The evidence for a direct relationship between DNA strand breaks and inhibition of replicon initiation is equivocal and the effect of DNA damage on DNA chain elongation has proved to be difficult to analyse. At least in the case of ultraviolet-irradiated cells DNA synthesis is initially blocked at damaged sites but it eventually continues past these lesions. The role of this physical inhibition of replication is therefore not clear.

It is difficult to study loss of gene function leading to metabolic disruption and cell death. The assumption has to be made that cell death by lethal mutations is a result of similar processes to mutation studied in easily analysed systems (e.g. drug resistance). The only difference is the relative importance to normal cell function of the gene loci under scrutiny. In analysing these systems it has become evident that irradiated cultures [17] can have a reduced growth rate thus supporting the potential growth inhibiting effects of radiation-induced mutations. There is no doubt that radiation can lead to significant loss of genetic material and that this leads to loss of function of some genes. It is therefore reasonable to suggest that some cell death will be due to the loss of function of essential genes.

Cells may differ in their tolerance of residual DNA damage

The ultimate test of the importance of residual damage comes in the investigation of the relationship between residual damage and cell death. This has been done rarely but a classic study of this comes from micronucleus formation in Syrian hamster cells [18]. In the pure diploid parent line fragment loss, as detected by micronucleus formation, related closely to impaired (slow growth) or inhibited colony formation. A spontaneous tetraploid variant was much less sensitive to fragment loss and a hypotetraploid variant was even more resistant to this loss. The implications of this for tumour therapy are obvious when one considers the high proportion of tumours which are aneuploid. The explanation of these results may lie in part in a simple effect of hyperdiploid cells being resistant to fragment loss because they have more copies of genes to start with. But this is not the sole answer.

It is well described in prokaryotic cells irradiated with UV that DNA replication can bypass DNA lesions. A stop in replication at the damaged site, continued replication beyond that site then gap filling by a recombination process is one model which has been proposed to explain this [19]. Analysis of this tolerance of DNA damage in mammalian cells is fraught with difficulties and evidence for it is yet to come.

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