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Mini-review

# Direct and bystander radiation effects: A biophysical model and clinical perspectives

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ABSTRACT

In planning treatment for each new patient, radiation oncologists pay attention to the aspects that they control. Thus their attention is usually focused on volume and dose. The dilemma for the physician is how to protract the treatment in a way that maximizes control of the tumor and minimizes normal tissue injury. The initial radiation-induced damage to DNA may be a biological indicator of the quantity of energy transferred to the DNA. However, until now the biophysical models proposed cannot explain either the early or the late adverse effects of radiation, and a more general theory appears to be required. The bystander component of tumor cell death after radiotherapy measured in many experimental works highlights the importance of confirming these observations in a clinical situation.



## 1. Effects of radiation exposure on biological systems

Irradiation of any biological organism generates a series of processes that differ enormously in a time-scale. The first step consists of interactions between photons, or particles, belonging to the radiation beam and the atoms the organism is made up of. As the radiation tries to penetrate the tissues, it will collide with atoms. Occasionally the collision will be so violent, mainly with innermost electrons, that some of the electrons may be ejected and become free. Ionization occurs because an ion pair has been created. The principal damaging effect of radiation arises from its ability to eject electrons from molecules within the cells, thus causing damage to all the molecules in the cell. This step, characterized by the process of energy transfer leading to ionizing and excitation of atoms and the breakage of chemical bounds, is centred on the formation of broken molecules and free radicals. The vast majority of lesions in DNA are successfully repaired. Some rare lesions fail to repair leading to eventual cell death. Some lesions are more serious than others and the prevalent hypothesis is that the mechanism of radiation-induced cell killing identifies DNA as the most important sub-cellular target at biologically relevant doses, and its double strand-break (DSB) as the most severe lesion.

Whilst historically the main application of radiation chemistry of relevance to radiation biology has been research into radiation-induced DNA damage, and its chemical characterization, it is interesting to note that radiochemical processes after this energy deposition lead to altered DNA molecules. The variety of DNA lesions induced by radiation is described on a scale of lesion severity. Severity includes not only the physical size of the DNA lesion but also its reparability [1]. After a repair interval of a few hours, the unrepaired lesions will predominantly be those with the highest severity. Within this category, we include DSBs that have not been rejoined as well as those that have been mis-repaired; a small proportion of these DSBs eventually lead to cell death [2]. In general, DSBs are produced in proportion to dose by two main processes:

(A) *Direct action:* There are DSBs resulting from a single energy deposit that simultaneously produces the lesion in both DNA strands and also lesions produced by a cluster of events in the DNA [3]. Another possible mechanism of direct action is that one DSB could be the result of two or more different energy deposit events, and the accumulative action of sub-lethal damage might lead to local multiple damage sites [4].
(B) *Indirect action:* There are DSBs that result from a single free-radical attack [5]. Evidence indicates that this process is the dominant mechanism of DSB formation with low LET radiation [6,7].

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There is compelling evidence to suggest that the DNA DSBs are the most important lesion causing chromosomal aberrations and cell lethality [4]. Direct action is mainly dependent on the physical features of the interaction process, such as the properties and quality of radiation (lineal transfer energy, LET) and the size of the target (DNA) which is constant for all human cells. Therefore, it seems reasonable to consider the number of DSBs produced by this pathway is constant. On the other hand, it has been proposed that the probability of DNA breakage by oxidative stress at a given site is mainly determined by the intrinsic structure of the double helix [8]. It should be remembered that oxygen radical action is a competitive process against the capacity of cells to neutralize the toxic effect of free radicals, i.e., their scavenging potency [9]. Therefore, a prominent feature of radiation biology is that the chemical condition of the DNA environment has an important influence on radiosensitivity [10–12] whereas the non-scavengeable DSBs could be due to direct effects [13]. In general non-scavengeable DNA-damage can be separated into two major groups: DSBs and non-DSB oxidative clustered DNA lesions [14]. Theoretically, 30–40% of DNA DSBs generated by radiation are due to direct action mechanisms [6]; the remaining 60–70% may be determined by the biological properties of the cells, including their scavenging capacity, chromatin conformational status, proliferative activity, differentiation grade and/or cell cycle phase [9,10,15]. While isolated damage is generally repaired efficiently, clustered DNA lesions have been suggested to be more difficult to repair [16].

The dose-rate effect in mammalian cells is seen as a change in the extent of cell killing when radiation is given at dose rates over the range 0.01–1.0 Gy/min. The interpretation of such results has shown that at a low dose rate repair can occur during irradiation, thus increasing survival. However, such dose-rate sparing is never complete, and this has led to the suggestion that two types of damage are inflicted by ionizing radiation, one that is irreparable and another that is reparable but which may not be reparable if fixed by mis-repair or binary interaction [1,17,18].

Previous contributions of radiation chemistry to radiation biology are unmistakable, but there remains considerable potential to help advance the biological understanding using the knowledge and techniques of radiation chemistry [16,19].

## 2. The three steps of radiobiology: transfer, communication and consequence

The irradiation of any biological system triggers a series of processes that are very widely separated in time. The ***Transfer*** of energy consists of interactions between the radiation and the atoms that make up the biological system. This step is characterized by the appearance of ionisations, excitations, bond-breaking and free radical formations.

In response to damage produced by ionising radiation, cells activate a series of biochemical processes, ***Communication***, that initiate the chain of signals of damage induced by genotoxic agents. The DNA damage response (DDR) is a highly complex and coordinated system that determines the cellular outcome of damage caused by radiation. Communication enables cells to increase their likelihood of survival by maintaining the integrity of the genome and its corresponding activities. This requires activation of the cell defence system, which consists of two parts, (a) the sensors of DNA damage and (b) the effectors of damage response.

Most of the lesions produced by radiation are efficaciously repaired. Some lesions cannot be repaired and cause the death of the cell, or can be tolerated remaining as residual damage. A time reference is necessary: cells take time to die, and irradiated cells are even able to divide once, or more times, before finally disappearing [20]. Radiotherapy, like any other cytotoxic agent, profoundly disturbs the balance between cell proliferation and cell death in both tumor and normal tissues, through the radiation induced DNA damage, increasing the likelihood of cell death by apoptosis, necrosis, autophagy or mitotic catastrophe. The DDR determines not only the sensitivity of cells to die following radiation, but also the type of cell death that occurs, and the timing of cell death.

Radiotherapy is highly effective in killing cells. The eradication of all the clonogenic cells from a tumor leads to cure, and this is the therapeutic end-point. What we have designated the ***Consequence*** is the result of two factors: (i) the probability of tumor control and (ii) the probability of inducing severe complications in healthy tissues. As with any other medical procedure, prescription of a course of radiotherapy must represent a balance between risk and benefit, the relative weight of which will determine the therapeutic gain. It is our hope that in the near future medical application of biotechnology will produce reliable assays for measuring these two variables, so we will be able to use them with a high degree of certainty.

### *2.1. Transfer: from the molecular DNA damage to the differences in radio-sensitivity*

When cells are irradiated, three molecular end-points have been identified which often correlate with cellular radiosensitivity:

(i) cells may vary in the amount of damage induced by a given dose of radiation [21–24];
(ii) cells show a different capacity, fidelity and rate to repair radiation damage to DNA [23,25–27]; and
(iii) cells differ in the level of residual damage after repair of the initial damage and recovery of cells [23,28–30].

Evidently, these three mechanisms may be linked through a common factor such as the modulation of chromatin conformational structure [31–35].

The transfer of energy to the cells takes place for the time required by the radiation to pass through the cell ($t < 10^{-13}$ s). The initial ionizations and excitations are followed by a series of physical–chemical processes at the centre of which is the formation of free radicals. The half-life of a free radical is $10^{-5}$ s. The amount of DNA damage produced in this time is inversely related to the survival of the treated cell measured as intrinsic radio-sensitivity using a clonogenic survival assay [21,24,36,37].

The number of DBSs induced in DNA from irradiated cells is believed to be related to the fraction of DNA fragmented under a given threshold size and is thus able to migrate under pulsed field gel electrophoresis [38,39]. Based on this fact we devised a method to calculate the number of DNA DSBs [21,24]. The final equation of the mathematical model has been published in another paper [40].

However, in the sequence of processes that follow the initial events in the energy transfer step and eventually lead to cellular and tissue consequence, there are a large number of poorly understood processes [41]. In response to ionizing radiation, cells immediately activate the sensors of DNA damage that actively survey the genome for the presence of damage. These proteins then signal the three main effector pathways that together determine the outcome for the cell: DNA repair, damage checkpoints and programmed cell death.

### *2.2. Communication: sensing and signaling DNA damage*

DNA repair mechanisms in cells are highly efficient and the vast majority of the lesions are normally repaired satisfactorily. A key unresolved issue is how a cell determines when DNA damage is