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Review Article

Radiation-induced clustered DNA lesions: Repair and mutagenesis☆

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ABSTRACT

Clustered DNA lesions, also called Multiply Damaged Sites, is the hallmark of ionizing radiation. It is defined as the combination of two or more lesions, comprising strand breaks, oxidatively generated base damage, abasic sites within one or two DNA helix turns, created by the passage of a single radiation track. DSB clustered lesions associate DSB and several base damage and abasic sites in close vicinity, and are assimilated to complex DSB. Non-DSB clustered lesions comprise single strand break, base damage and abasic sites. At radiation with low Linear Energy Transfer (LET), such as X-rays or γ-rays clustered DNA lesions are 3–4 times more abundant than DSB. Their proportion and their complexity increase with increasing LET; they may represent a large part of the damage to DNA. Studies *in vitro* using engineered clustered DNA lesions of increasing complexity have greatly enhanced our understanding on how non-DSB clustered lesions are processed. Base excision repair is compromised, the observed hierarchy in the processing of the lesions within a cluster leads to the formation of SSB or DSB as repair intermediates and increases the lifetime of the lesions. As a consequence, the chances of mutation drastically increase. Complex DSB, either formed directly by irradiation or by the processing of non-DSB clustered lesions, are repaired by slow kinetics or left unrepaired and cause cell death or pass mitosis. In surviving cells, large deletions, translocations, and chromosomal aberrations are observed. This review details the most recent data on the processing of non-DSB clustered lesions and complex DSB and tends to demonstrate the high significance of these specific DNA damage in terms of genomic instability induction.

1. Introduction

About two third of the DNA damage induced by low linear energy transfer (LET) radiation (e.g., X-rays and γ-rays) are formed by reactive oxygen species, including the highly damaging hydroxyl radical, produced by water radiolysis in the vicinity of DNA [1,2]. Thus, ionizing radiation induces random (isolated) single strand breaks (SSB), oxidation reactions to the sugar moieties which partly lead to the formation of SSB, oxidized/reduced base damage, base loss, many of which do not chemically differ from that formed during endogenous oxidative metabolism or by oxidizing agents. Meanwhile, at equal number of oxidatively generated DNA damage, ionizing radiation is far more toxic than hydrogen peroxide [3,4]. Modeling of the radiation track structures [1,5] evidenced that the energy deposition of low LET radiation leads to two or more ionizations within a radius of 1–4 nm, like the diameter of the DNA double helix and its water layers. Multiple ionizations result in multiply damaged sites, e.g. clustered DNA lesions, which consists in > 2 SSB, abasic (AP) sites, oxidized purine or pyrimidine bases, or double strand breaks (DSB), formed within one or two helix turns from the same energy deposition event. Overall, ionizing radiation, like oxidative metabolism, produces isolated SSB, AP sites, oxidatively generated base damage, but also produces DSB, DNA protein crosslinks and clustered DNA lesions (Table 1) which are rarely formed by endogenous oxidative stress but recognized as largely responsible for the toxicity of ionizing radiation. High LET radiation (e,g., α-particles, protons and carbon-ions such as used in hadrontherapy) has an elevated propensity to form clustered DNA lesions of higher complexity in comparison with low LET radiation. This is well schemed

Abbreviations: LET, linear energy transfer; MDS, multiply damaged site; SSB, single strand break; DSB, double strand break; AP sites, abasic sites; 8-oxoG, 8-oxo-7,8-dihydroguanine; 5-ohU, 5-hydroxyuracil; Tg, 5,6-dihydroxy-5,6-dihydrothymine; U, uracil; BER, base excision repair; NHEJ, non-homologous end joining; HR, homologous recombination; bp, base pair; IRIF, ionizing radiation-induced foci

☆ This article is one of a series of papers on the subject of oxidative DNA damage & repair that have been published as a special issue of Free Radical Biology & Medicine to commemorate the Nobel Prize won by Prof. Tomas Lindahl. A detailed introduction and synopsis of all the articles in the special issue can be found in the following paper by Cadet & Davies [129].

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Table 1
DNA damage induced by ionizing radiation.[a]

Type of damage	Radioinduced damage per cell per Gy	Endogenous damage per cell per day
Single strand breaks	1000	> 10000
Base damage	2000	3200
Abasic sites	250	12600
Double strand breaks	40	40–50[c]
DNA-protein XL	150	?
Non-DSB clustered lesions	122[b]	?
Complex DSB	?	

[a] From [126–127].
[b] From [14,29].
[c] From [128].

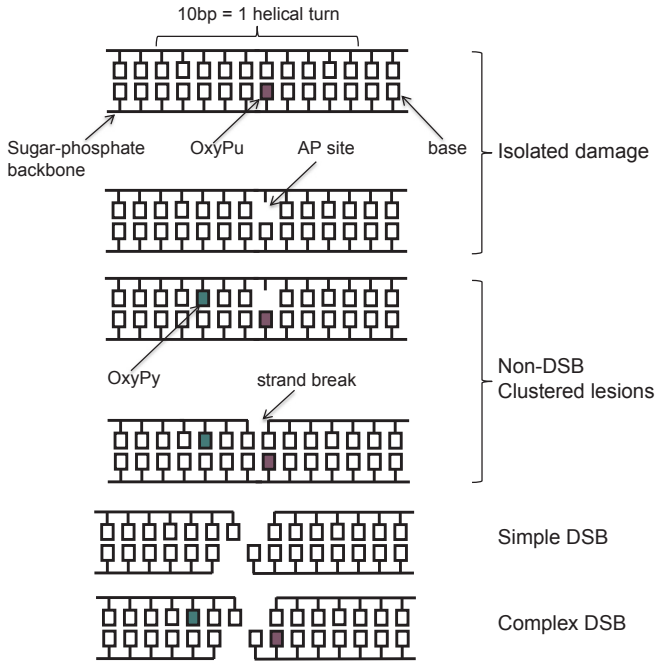

Fig. 1. Scheme of oxidatively generated DNA damage induced by ionizing radiation. Non-DSB clustered lesions comprises 2, 3 or more oxidatively generated base damage (noted oxyPu, oxyPy, U), abasic site, SSB within one or two helix turns of DNA, produced by a single radiation track, the complexity of which increases with increasing LET. Complex DSB are DSB associated with several oxidized bases and abasic sites.

in a recent review by Georgakilas et al. ([6]; see Fig. 2 of the cited review). In fact, high LET radiation shows a greater relative effectiveness than low LET radiation regarding many cellular end points and they are more harmful.

Clustered DNA lesions, as soon as they were predicted by chemists and physicists, were suspected to have a low ability to be repaired, due to the peculiar spatial distribution of the lesions [1,5]. In addition, for the same reason, they cannot be measured by the analytical methods used for isolated oxidatively generated lesions, and until now there is no reliable methods for their precise quantification. The first observation was made in irradiated plasmid DNA [7], extended by Sutherland findings in phage DNA and mammalian cells [8]. Nevertheless, a good estimation was first provided by Sutherland and coworkers, who demonstrated that non-DSB clustered lesions (Fig. 1), carrying oxidized purine or pyrimidine bases, AP sites or SSB, comprise the majority (70–80%) of clustered DNA lesions in mammalian cells and are 3–4 times more frequent than prompt DSB by low LET radiation [8–12]. Isolated oxidatively generated DNA lesions (SSB and oxidized base) are known to be efficiently and accurately repaired by base excision repair (BER) which is largely reviewed in this issue. Radiation-induced simple prompt DSB, although carrying 3'-ends phosphate and phosphoglycolate that require processing (in contrast to DSB with ligatable ends produced by restriction enzymes) can also be efficiently and accurately repaired within 1–2 h by non-homologous end joining (NHEJ), or in G2 phase by homologous recombination (HR), in mammalian cells. A small fraction of radiation-induced DSB, and in particular those induced by high LET radiation, often called complex DSB, are known to have a slow rate of repair and to require DNA damage response *via* the ATM pathway [13]. Sutherland and collaborators have reported that the non-DSB clustered lesions produced in mammalian cells by radiation show a greatly longer lifetime than that of isolated DNA lesions [8,10,14]. The last two decades the strategy that consists in building synthetic non-DSB clusters of various complexity, carried on oligonucleotides, and in using them to study DNA repair in test tubes or in bacterial, yeast or mammalian cells, has greatly helped understanding the processing of clustered DNA lesions at the molecular level (reviewed in [4,15–18]). Indeed, studies of survival, mutation induction and repair of/in plasmid carrying non-DSB clusters of various nature and complexity, in *Escherichia coli*, yeast and mammalian cells have been of utmost importance.

An attempted simultaneous repair of opposed base damage or AP sites at non-DSB clusters may result in the formation of DSB [4]. Measuring DSB post-irradiation in mammalian cells and bacteria has revealed an increase in DSB when cells were allowed time for repair [19–21]. Such a DSB produced as repair intermediate may be surrounded by 1–2 unrepaired base damage and become an unrepairable, complex DSB. Alternatively, a non-DSB cluster may be resistant to glycosylases and endonucleases and the persistent lesions within the cluster may lead to mutations. Clustered DNA lesions, including DSB and non-DSB clusters, challenge the DNA repair and the outcome of a cell. They are considered as responsible for the genomic damage and instability inflicted by ionizing radiation. This mini-review will focus on the most recent aspects of the formation, repair and biological consequences of clustered DNA lesions induced by ionizing radiation.

2. Formation, detection and structural features of clustered DNA lesions

Clustered DNA lesions are considered as a hallmark of ionizing radiation exposure, as their endogenous production in cells is very low [22,23] and due to their mode of formation. The simplest clustered DNA lesion is the DSB (2 opposed SSB in a close vicinity). Complex DSB and non-DSB clusters can carry as many as 10 lesions per damaged site for low LET radiation, and more for high LET radiation, according to theoretical calculation [24]. Indeed, the average number of lesions per cluster tends to increase with increasing LET. For example, 1 MeV Protons (LET 25,4 keV/μm) are predicted to produce DNA lesions with a ratio of 1 five-lesion cluster: 20 two-lesion clusters: 60 isolated lesions, whereas 4 MeV α-particles (LET 105 keV/μm) are predicted to produce DNA lesions with a ratio of 1 five-lesion cluster: 4 two-lesion clusters: 8 isolated lesions [25]. Monte Carlo track structure simulations of ionizing particles, also indicated that about 30% of DSB are complex DSB with at least one lesion (including base damage) in close proximity at low LET, whereas this is 90% at high LET [25]. It was also predicted 3–4 times more non-DSB clusters than DSB at low LET, and up to 8 times more at high LET [25]. This was confirmed by experimental determinations for low LET radiation [8,12]. However, Hada and Sutherland [26] reported that the relative frequencies of DSBs compared to bistranded abasic and oxidized base clusters were higher for the charged particles than for X-rays, in (linear) T7 phage DNA. Extended and refined Monte Carlo track structure simulations recently calculated that complex SSB associated with base damage represent 49% of all SSB for low LET radiation, and 87% for the high LET radiation tested. In addition, calculations showed that nearly all DSB