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Non-DSB clustered DNA lesions. Does theory colocalize with the experiment?

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HIGHLIGHTS

- Ionizing radiation induces clusters of DNA damage at nano- scale.
- Monte Carlo simulations predict the ionization clustering.
- Different methodologies detect non-DSB clustered DNA lesions at nano- or micro-scale.
- There is often a disparity between the theory and experimental evidence.
- Fluorescence microscopy can be used for discovering damage complexity in situ.

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ABSTRACT

Ionizing radiation results in various kinds of DNA lesions such as double strand breaks (DSBs) and other non-DSB base lesions. These lesions may be formed in close proximity (i.e., within a few nanometers) resulting in clustered types of DNA lesions. These damage clusters are considered the fingerprint of ionizing radiation, notably charged particles of high linear energy transfer (LET). Accumulating theoretical and experimental evidence suggests that the induction of these clustered lesions appears under various irradiation conditions but also as a result of high levels of oxidative stress. The biological significance of these clustered DNA lesions pertains to the inability of cells to process them efficiently compared to isolated DNA lesions. The results in the case of unsuccessful or erroneous repair can vary from mutations up to chromosomal instability. In this mini review, we discuss of several Monte Carlo simulations codes and experimental evidence regarding the induction and repair of radiation-induced non-DSB complex DNA lesions. We also critically present the most widely used methodologies (i.e., gel electrophoresis and fluorescence microscopy [in situ colocalization assays]). Based on the comparison of different approaches, we provide examples and suggestions for the improved detection of these lesions in situ. Based on the current status of knowledge, we conclude that there is a great need for improvement of the detection techniques at the cellular or tissue level, which will provide valuable information for understanding the mechanisms used by the cell to process clustered DNA lesions.

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Abbreviations: BER, Base excision repair; B-NHEJ, backup NHEJ; DDR, DNA damage response; D-NHEJ, referred as canonical or classical NHEJ; DSBR, double-strand break repair; DSBs, DNA Double strand breaks; F_{like}, Foci-like analysis parameter; IR, Ionizing Radiation; HR, Homologous recombination; LET, Linear energy transfer; LMDS, Locally Multiply Damaged Sites; MC, Monte Carlo; MCNP, Monte Carlo N-particle; MOC, Manders overlap coefficient; NALA, Number average length analysis; NER, Nucleotide excision repair; NHEJ, Non-homologous end joining; NIR, near infrared radiation; OCDLs, oxidative clustered DNA lesions; PCC, Pearson's correlation coefficient; P_{clc}, co-localization Parameter; ROS, reactive oxygen species; RIF, radiation-induced foci; RT, Radiation therapy; RBE, Relative biological effectiveness; SSBs, Single strand breaks

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1. Introduction

In cancer treatment, radiation therapy (RT) uses ionizing radiation (IR) as a cancer cell killing agent. The ability of IR to kill cells is based on the nature of its interaction with biological matter or organisms. Based on accumulating theoretical (Monte Carlo-MC-simulations) and experimental evidence, the specific fingerprint of IR is the induction of DNA damage resulting from the physical and chemical interactions at the nano- or micro- scale (Lorat et al., 2015). Specifically, we refer to the well-known interactions of photons or charged particles with atoms and molecules when traversing biological hydrated material. During this photon or ion track, direct deposition of energy (elastic and inelastic collisions with genomic DNA) and creation of secondary electrons is expected. This specific interaction pattern of IR and especially of charged particles results in the induction of highly localized ionization events (clusters) and consequently closely spaced DNA lesions (i.e., the so called 'clustered or complex' DNA damage) (Hada and Georgakilas, 2008; Held et al., 2016). These densely induced lesions (double strand breaks [DSBs], single strand breaks [SSBs], abasic sites [AP], and oxidized base lesions) occur within a small fragment of DNA, up to 2 helical turns in length, and in most cases by a single radiation track. These clustered DNA damages have also been detected at a larger scale like kbp or Mbp chromosomal area using innovative electrophoretic or immunofluorescence colocalization techniques, respectively (Sutherland et al., 2000a; Asaithamby et al., 2011). They are considered the fingerprint of IR since DNA lesions occurring spontaneously as a result of intrinsic cellular processes are homogeneously distributed and usually not in a clustered formation (Nikitaki et al., 2015a). Furthermore, the DNA damage spectrum depends on the incident radiation (Hada and Sutherland, 2006). A typical cluster may contain for example a DSB and some base lesions or a SSB 'marking', which makes this DNA region difficult to be repaired and therefore at high risk for developing mutations (Eccles et al., 2011) and chromosomal breaks (Asaithamby et al., 2011). As the processing of clustered DNA damage is crucial for cell survival, genomic instability induction is most likely to happen if the repair process is not successfully completed or delayed (Georgakilas et al., 2004; Tsao et al., 2007). This pattern of oxidative clustered DNA lesions (OCDLs) has been also mentioned to occur not only in the cases of IR but also UV and near infrared (NIR) femtosecond laser microbeams (Botchway et al., 2012; Greinert et al., 2012).

In this review, we discuss the current status of knowledge in the field of theoretical and experimental approaches relating to the induction and measurement of clustered lesions (DSBs and non-DSB) by IR with emphasis on the discrepancies between the different methodologies for detection of clusters at the cellular level. Finally, we discuss whether the DNA lesions predicted by the theory (MC models) are verified or not by the experiments.

2. Monte Carlo simulations for DNA damage calculations

2.1. Historical perspective of Monte-Carlo simulation codes for DNA damage calculations

For doses up to $\sim$1 Gy it can be estimated that, on average, 1 (or at most 2) independent gamma-radiation tracks traverse a cell-like volume equal to a γ-H2AX focus ($\sim$500 nm diameter). As one focus corresponds to one DSB (to a good approximation, at least for low doses $\leq$1 Gy and low-LET radiations) (Sedelnikova et al., 2002), it can be argued that foci formation is triggered by single-track effects at the DNA level (1–10 nm). Although the γ-H2AX methodology is considered the most sensitive towards the detection of DSBs, there is always a possibility of underestimating DSBs especially when comparing to electrophoretic approaches. In general, the number of foci is less than the number of DSBs detected by agarose gel electrophoresis. Even for low-LET radiations, there is still a probability of existence of more than one DSBs 'under' one single γ-H2AX focus (DSB complexity). Recent theoretical, simulation and experimental evidence, suggests that the possibility of finding two or more DSBs under one γ-ray induced focus is very low for doses below 1 Gy ($<$1 Gy). For high-LET α-particles though and based on Monte Carlo simulations, $\sim$8 DSBs are expected to be induced per track and practically under each focus (Antonelli et al., 2015). On the other hand, one cannot disregard, the case of overestimating prompt DSBs using for example pulsed field gel electrophoresis due to the conversion of thermally or alkali labile sites into DSBs (Singh et al., 2013; Singh et al., 2011).

The ability of radiation tracks to form clustered lesions at the DNA level, can be attributed to the spatial distribution of energy deposition in matter (Nikjoo et al., 1998). In particular, the stochastic and inhomogeneous pattern of radiation interactions plays a key role in the induction of DSBs and, more generally, of Locally Multiply Damaged Sites (LMDS) as first introduced by John Ward (Ward, 1981, 1985). Revolutionary for their time, MC studies supported this IR-induced clustering for low- or high-LET radiation with specific of course spatiotemporal differences expected for radiation of different quality (Nikjoo and Goodhead, 1991; Nikjoo et al., 1994). This is in sharp contrast to the tissue (or organ) level where nonstochastic quantities (like absorbed dose) averaged over macroscopic volumes usually provide a reliable physical description of biological effects (Rossi and Zaider, 1996). The effect of the scaling used for micro and nanodosimetry is also discussed in Plante et al. (Plante et al., 2013).

Detailed calculations and MC simulations by Goodhead and Nikjoo through the years have suggested that the amount of energy deposited by interaction of electrons in small target volumes, such as comparable to the dimensions of DNA like a nucleosome or a short chromatin segment is quite limited (Goodhead, 1989; Goodhead and Nikjoo, 1989). The most relevant frequency distributions for X-rays would be those for the 100 keV electrons. The energy deposition distributions can be roughly converted into distributions of number of ionizations in liquid water by assuming about 20–25 eV per ionization event on average. For example, 100 eV deposited in a target volume would correspond on average to 4–5 ionizations. Based on the above studies and an overall analysis of different studies (Georgakilas et al., 2013), one can safely assume, that only a small proportion of ionizations is in sufficient proximity to the DNA to lead to strand breakage or base damage, either by direct ionization of the DNA or by reaction of diffusing hydroxyl radicals (·OH) created by the radiolysis of water. Here we should note, that the relatively small ($\sim$4 nm) diffusion distances of .OH in the highly reactive environment of a cell does not really support the idea for an electron track to produce 2 strand breaks and also additional DNA damage (Nikjoo et al., 1997). It is generally suggested that the percentage of DSBs that have one or more associated damaged bases may be of the order of 60% for the very low energy electrons and even smaller for hard X-or γ-rays (40% to 50%) (Nikjoo et al., 1999). Recent MC studies agree with earlier findings (for both low- and high-LET radiations) suggesting that the ratios of the yields of base damage to SSBs are about 3 and the average SSB/DSB ratio for low-LET radiations is about 18, which is about 5 times higher than the corresponding for high-LET radiations (Watanabe et al., 2015). Experimental evidence suggests similar ratios (reviewed in Georgakilas et al. (2013) and Hada and Georgakilas (2008)) with of course a great dependence on variations in the experimental system (type of cells, DNA, oxygen percentage) and methodology followed to measure the non-DSB lesions (Nikitaki et al., 2015a).

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