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The repair of environmentally relevant DNA double strand breaks caused by high linear energy transfer irradiation – No simple task

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ABSTRACT

High linear energy transfer (LET) ionising radiation (IR) such as radon-derived alpha particles and high mass, high energy (HZE) particles of cosmic radiation are the predominant forms of IR to which humanity is exposed throughout life. High-LET forms of IR are established carcinogens relevant to human cancer, and their potent mutagenicity is believed, in part, to be due to a greater incidence of clustered DNA double strand breaks (DSBs) and associated lesions, as ionization events occur within a more confined genomic space. The repair of such DNA damage is now well-documented to occur with slower kinetics relative to that induced by low-LET IR, and to be more reliant upon homology-directed repair pathways. Underlying these phenomena is the relative inability of non-homologous end-joining (NHEJ) to adequately resolve high-LET IR-induced DSBs. Current findings suggest that the functionality of the DNA-dependent protein kinase (DNA-PK), comprised of the Ku70-Ku80 heterodimer and the DNA-PK catalytic subunit (DNA-PKcs), is particularly perturbed by high-LET IR-induced clustered DSBs, rendering DNA-PK dependent NHEJ less relevant to resolving these lesions. By contrast, the NHEJ-associated DNA processing endonuclease Artemis shows a greater relevance to high-LET IR-induced DSB repair. Here, we will review the cellular response to high-LET irradiation, the implications of the chronic, low-dose modality of this exposure and molecular pathways that respond to high-LET irradiation induced DSBs, with particular emphasis on NHEJ factors.

1. Ionising radiation exposure

DNA damage is a constant threat to genomic integrity and may arise endogenously or be induced exogenously by environmental mutagens such as ionising radiation (IR) [1]. Perhaps the most toxic type of IR-induced DNA lesion is the DNA double-strand break (DSB), formed when both strands of the phosphodiester DNA backbone are broken. Potentially lethal to cells at even a singular occurrence, DSBs are generally detected and repaired with great speed, with Non-Homologous End-Joining (NHEJ) serving as the primary means of DSB repair for the majority of IR-induced lesions [2,3]. Failure or even partial insufficiency of NHEJ has been observed to correlate strongly with eukaryotic radiosensitivity, underlying its importance to terrestrial life.

For humanity, exposure to at least some IR throughout life is unavoidable. The majority of the average individual's annual IR exposure is in the form of higher linear energy transfer (LET) radiation, such as highly charged, energetic (HZE) particles within cosmic radiation or alpha (α) particles emitted by decaying radon gas of geological origin [4]. LET is used to describe the rate at which energy is released by a radiation source over a fixed distance, with high-LET radiation emitting more energy than low-LET over the same distance [5]. Collectively, high-LET sources account for a majority (~65%) of our annual IR exposure and are almost entirely of natural origin [4]. Lower LET radiation, such as gamma (γ)- or X-rays, account for our remaining exposure, split between artificial (medical, industrial or military) and natural (buildings, geology, etc.) sources. With the exception of certain artificially sourced irradiation events (which are often acute and at higher doses), most of our lifetime IR exposure occurs at low to very low doses and chronically (i.e. over a long period of time). Despite this, historically the majority of studies into the eukaryotic response to IR exposure, and NHEJ in particular, have utilized low-LET radiation sources (γ- or X-rays) delivered acutely at relatively high dosage. While these low-LET IR studies have been foundational to our knowledge of DSB repair and radiation biology, there is an increasing realization that high-LET IR-induced DNA damage, to which we are most frequently exposed during life, has distinct genetic and molecular requirements to resolve [6]. This area is now subject to increasing interest as researchers take aim at resolving the barriers posed by high-LET space radiation to extraterrestrial exploration and colonization, combating high worldwide incidences of radon gas related

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lung cancer, assessing the long-term impact of nuclear disasters such as the 2011 Fukushima nuclear plant meltdown and the utility of high-LET IR as anti-cancer therapy. Here, we will review our current understanding of the molecular pathways that respond to high-LET irradiation induced DSBs, with particular emphasis on NHEJ.

2. Radiation linear energy transfer, DNA damage complexity and repair

A definition commonly used to describe the impact of different qualities of IR is relative biological effectiveness (RBE), the dose ratio of low-LET to high-LET IR needed to produce the same biological effect. For high-LET neutrons, the RBE is 2–4 relative to low-LET X-rays; in other words, the high-LET IR is 2–4 times more effective at killing cells than an identical dose of low-LET IR [5]. In terms of human health, high-LET IR is classified by many authorities, including the United Nations World Health Organization, as both highly carcinogenic and dangerous to life [4,7].

2.1. Radiation LET and clustered lesions

In its most uncomplicated form, a DSB represents two opposing DNA ends with an easily ligatable 5′-phosphate and a 3′-hydroxyl. Such simplicity is produced in abundance by DSB-inducing restriction enzymes, but is believed to represent the minority of lesions after IR exposure, where increasing collateral damage to the DSB termini (5′-hydroxyls, 3′-phosphates, phosphoglycolates, protein-adducts, etc.) or surrounding bases (apurinic/apyrimidinic (AP) sites, intra- and inter-strand crosslinks, etc.) is more common; such lesions are often referred to colloquially as 'dirty' or 'complex' and increase in frequency with increasing LET of the IR [8]. Underlying this phenomenon is clustering, the relative proximity of one DSB or other type of DNA lesion relative to another. Higher LET IR equates with greater lesion clustering due to the confined nature of the energy deposition; for example, α-particles have a very high energy (five million electrovolts per particle) but a very short range, depositing ~90% of their energy within a 10 nm radial spread and producing DSBs spaced only about 10–20 bp apart [9,10]. Within that confined space, direct ionization events along the DNA backbone will generate closely spaced DSBs and reactive free radicals leading to strand cross-linking [6] (Fig. 1). Local water ionization will generate reactive oxygen species (ROS) that may also react with bases or the phosphodiester backbone to generate yet more DSBs in a confined area [6]. While lower LET IR will also produce the same spectrum of lesions, these will be more widely spaced, and may only have a small number of nearby crosslinks or base damages in close enough proximity to interfere with DSB repair [6,10].

The idea of clustered DNA damage was first described in the 1980's as locally multiple damaged sites (LMDS) [11,12]. Clustered DNA damage is now defined as two or more lesions formed within one or two helical turns of DNA caused by the passage of a single radiation track [13–15]. Theoretical systems have suggested that high-LET IR can create as many as 25 lesions per 1–2 helical turns [16]. This finding is supported by studies modelling DNA damage following traversal by varying IR particles, which suggest that at least 70% of DSBs are clustered with other DSBs for high-LET IR, dropping to only 30% for low-LET [6,10,17]. If other types of DNA damage are additionally accounted for, then >90% of high-LET IR-induced DSBs are considered clustered, versus only 60% induced by low-LET IR [17]. Of the other types of damage to be found in proximity to DSBs, AP sites were found to arise most frequently, within 8–10 bp of a DSB end [18]. Although also a direct consequence of IR exposure, interstrand crosslinks (ICLs) within clustered DSB lesions occur with a comparably lower frequency to AP sites, since they are thought to form only where mismatched bases or other non-hybridized DNA are present and ionized (for a review of IR-induced crosslinks, see [19]; for further discussion of IR-induced clustered DNA damage, also see [8,10]).

2.2. The challenges of repairing high-LET IR-induced DNA damage

DSBs induced by high-LET α-particles are known to have a slower rate of repair when compared to low-LET γ-radiation [20–22]. Pulse-field gel electrophoresis (PFGE) experiments suggest that while 90% of DSBs are repaired 3 h post 10 Gy γ-rays, only 50% of DSBs are resolved 3 h after an equivalent dose of α-particles. Even 1 day later, 30% of α-particle induced-DSBs remain, with 2–5% still present up to 10 days later [23]. Perhaps not surprisingly, greater cellular radiosensitivity following α-particle exposure is observed in comparison to γ-rays by clonogenic survival assay [24]. Although, dose-for-dose, both high- and low-LET IR produce similar DSB numbers, the closely packed DSBs caused by high-LET IR are more likely to result in small double stranded DNA (dsDNA) fragments compared to low-LET IR [25,26]. Indeed, increasing amounts of small dsDNA were observed by PFGE following irradiation with charged particles of increasing LET and were not observed after low-LET IR exposure [27]. Using plasmid DNA in vitro, significantly increased production of short dsDNA fragments was detected following high-LET IR Argon ion exposure compared to low-LET γ-rays, with approximately 70% of fragments being 0–50 nm in size [28]. Short dsDNA fragments are problematic to DSB repair due to competitive interactions with DSB response factors [28], possible diffusion away from sites of rejoining, assumed loss of associated nucleosomes (and associated epigenetic marks necessary for repair) and a propensity for triggering mutagenizing events such as gene amplification [29].

In agreement with loss of dsDNA stretches from clustered DSBs, numerous studies have found that genetic mutation frequency increases with increasing LET [30,31]. Mutagenesis following high-LET IR is further exacerbated as clustered DSBs are more likely to be misrejoined to one another due to the extreme proximity of multiple DSB ends, with 50% of α-particle-induced DSBs repaired within 24 h have been observed to be rejoined incorrectly [23]. Since the probability of DSB misrejoining depends more upon DSB proximity (e.g. clustering) than the absolute number of DSBs per cell, this means that DSB misrejoining following high-LET IR is essentially *independent* of IR dose (DSB clustering being extremely frequent even at low high-LET IR doses); this contrasts to the more dose-dependent relationship between low-LET IR exposure and erroneous repair [23]. Much of the biological impact of high-LET IR is due to single damage tracks traversing separate chromosomes and thereby enabling chromosome arm translocation at μm distances [6]. FISH analysis has shown that simple exchanges between two chromosomes arise from a single point of damage, and therefore are linear with dose [32]. By contrast, complex chromosome aberrations, defined as >3 breaks within ~2 chromosomes, are induced characteristically following exposure to high-LET IR, even at very low doses; in support, FISH studies determined that 83% of chromosome exchanges following low-dose α-particle exposure are 'complex', compared to only 30% for high dose X-irradiation [9]. Looking specifically at chromosome 5, a separate study found that more aberrations were induced by 0.2 Gy of α-particles versus 1.5 Gy of γ-rays [33]. Collectively, these data highlight the potent mutagenicity of high-LET IR at almost any dose.

2.3. High-LET IR, chromatin structure and IR-induced foci

Higher order chromatin structure has been suggested to be responsible for the majority of non-randomly distributed DSBs after