

Radiation track structure: how the spatial distribution of energy deposition drives biological response.

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Abstract

Ionising radiation is incredibly effective at causing biological effects. This is due to the unique way energy is deposited along highly structured tracks of ionisation and excitation events, which results in correlation with sites of DNA damage from the nanometre to the micrometre scale. Correlation of these events along the track on the nanometre scale results in clustered damage, which not only result in the formation of DNA double-strand breaks (DSB), but also more difficult to repair complex DSB which includes additional damage within a few base pairs. The track structure varies significantly with radiation quality and the increase in RBE observed with increasing LET in part corresponds to an increase in the probability and complexity of clustered DNA damage produced. Likewise correlation over larger scales, associated with packing of DNA and associated chromosomes within the cell nucleus, can also have a major impact on biological response. The proximity of the correlated damage along the track increases the probability of miss-repair through pairwise interactions resulting in an increase in probability and complexity of DNA fragments/deletions, mutations and chromosomal rearrangements. Understanding the mechanisms underlying the biological effectiveness of ionising radiation can provide an important insight into ways of increasing the efficacy of radiotherapy, as well as the risks associated with exposure. This requires a multi-scale approach for modeling, not only considering the physics of the track structure from millimetre scale down to the nanometre scale, but also the structural packing of the

DNA within the nucleus, the resulting chemistry in the context of the highly reactive environment of the nucleus, along with the subsequent biological response.

Introduction

While exposure to ionising radiation (IR) can pose a potential health hazard, most notably cancer along with tissue reactions (deterministic effects) at high doses, it can also be beneficial when used for medical exposures, such as diagnostic imaging or radiation therapy. Radiotherapy has conflicting requirements of maximising the dose to the tumour to cure the patient while minimising the dose to the surrounding tissue to limit normal tissue toxicity. This is generally achieved using multiple intersecting radiation beams (or arcs) collimated such that they are conformal to the tumour. Although high energy x-rays are primarily used, in recent years there has been a significant increase in the use of proton therapy, and to a lesser extent carbon ion therapy, with their superior depth dose distributions. With a higher linear energy transfer (LET)¹, carbon ions also offer a number of radiobiological advantages with their significantly higher relative biological effectiveness (RBE)² and a reduced oxygen enhancement ratio (OER)³. While manipulating the physical dose distribution on the millimetre scale is a major consideration of treatment planning, it is only part of the story in determining the ultimate biological response, with energy deposition on the micrometre and nanometre scales playing a critical role. Understanding the mechanisms underlying the

¹ LET represents the average rate of energy loss along the track of an ionising particle.

² RBE is defined as the inverse ratio of the amount of energy of absorbed dose required to produce a given effect for a particular radiation required to produce a given effect to a given reference radiation (usually x-rays or γ -rays)

³ OER is defined as the ratio of the dose required when delivered under anoxic conditions, compared to the dose required under fully oxygenated conditions to achieve the same biological effect.

biological effectiveness of IR can provide an important insight into ways of increasing the efficacy of radiotherapy, as well as the risks associated with exposure.

Radiation track structure

IR deposits energy in highly structured tracks of ionisation and excitation events correlated in space and time. These tracks vary significantly for different types of radiation resulting in a modulation of biological efficiency. Charged particles will readily ionise the atoms and molecules they traverse because of their charge, producing secondary electrons. Photons (x-rays and γ -rays) interact within the body via a range of process, each leading to the production of an electron (or electron-positron pair) and it is via these electrons that energy is deposited within the cell. The fast electrons produced by photons interact as they slow down, producing further fast electrons and so on, resulting in a track of ionisation and excitation events along the path of these electrons as illustrated in figure 1a ($\sim 10^5$ ionisations per cell per Gy) with the majority of interactions occurring in water. These interactions result in the production of highly reactive reducing (e^-_{aq} and H) and oxidizing ($\cdot OH$) species along the track. These will subsequently diffuse and interact in competition with scavenging reactions, which will limit their lifetime.

Linear Energy Transfer (LET) is often used to describe the average rate of energy loss along the track of an ionising particle [1]. X-rays and γ -rays are often referred to as sparsely ionising (average LET of about $0.2 \text{ keV}/\mu\text{m}$ for ^{60}Co γ -rays), while tracks produced by ions such as α -particles are often referred to as densely ionising (LET of $107 \text{ keV}/\mu\text{m}$ for 4.0 MeV α -particles). The LET of clinically relevant high energy 250 MeV protons is low ($0.4 \text{ keV}/\mu\text{m}$) but increases as the proton slows down in the body ($4.7 \text{ keV}/\mu\text{m}$ at 10 MeV), while clinically used carbon

ions beams are medium to high-LET. For a wide range of biological end-points the RBE is observed to increase with increasing LET up to a maximum at around 100 - 200 keV/ μm then decrease at higher values [2-6]. It is important to note that for a given quality of radiation the value of RBE is not unique, but can vary significantly depending on the biological end-point being compared, the cell line/tissue/animal being studied, dose delivered and the reference radiation used.

DNA damage

DNA has been identified as a critical target of IR for a range of biological endpoints [7], with increased sensitivity observed in cells and animals with DNA-damage response (processing/repair) deficiencies [7-10]. As illustrated in figure 1b, DNA damage can be produced either by direct ionization or excitation of its constituent atoms, or indirectly as a result of interactions in surrounding atoms/molecules (predominantly water) resulting in free radicals, most notably hydroxyl radicals ($\cdot\text{OH}$), able to diffuse and react with DNA. Due to the highly reactive environment within the cell, the lifetime of these hydroxyl radicals is of the order of 4 to 9 $\times 10^{-9}$ s which limits their diffusion distance to just 6 to 9 nm [11], so damage is produced in close proximity to the original track.

The quantity of DNA lesions produced by 1 Gy of IR (table 1) is significantly smaller than the ~50,000 DNA lesions (including ~3,600 single-strand breaks (SSB)) produced per cell per day as a result of endogenous damage from normal metabolism process of the cells. While the endogenous damage is typically repaired with high fidelity by a range of DNA repair mechanisms, this is not the case for radiation induced lesions. What makes IR unique is its efficiency at inducing clustered DNA lesions (defined as two or more lesions within one or two

helical turns of DNA) at a biologically relevant frequency due to the correlation of multiple energy deposition events in space and time along the radiation track. This results in a wide spectrum of clustered lesions (figure 2) from individual tracks, with the frequency and complexity depending on radiation quality. It is important to note that due to the very short range of the indirect effect with the cell, DNA double-strand breaks (DSB) and other clustered DNA lesions are the result of a **single** track, with a dose of greater than $\sim 10^4$ Gy required for a second independent track to contribute [12] and so will not be affected by dose rate or fractionation.

Not all DSB are equal; this is particularly true with respect to IR induced DSB where the increased ionization density associated with increased LET results not only in an increase in the probability of clustered damage but also an increase in the complexity of this damage. Even x-ray induced DSBs are estimated to be 4 – 40 times more effective than simple DSBs produced as a result of hydrogen peroxide treatment [14].

It is possible to investigate the variation in the spectrum of DNA damage with radiation quality using Monte Carlo techniques; these not only model the spatial distribution of energy deposition events, but also have ability to follow subsequent diffusion controlled reaction of the water radicals formed and resulting DNA damage. The pioneering work of Nikjoo, Goodhead and collaborators have shown that even for low-LET radiation $\sim 20 - 50\%$ of DSB are complex by virtue of additional strand-breaks and/or base damage within 10 base pairs [15, 16]. The frequency and complexity of complex DSB was observed to increase with increasing LET (see figure 2c), with greater than 90% of DSB being complex for high-LET α -particles [17]. The increase in complexity of these clustered lesions, such as DSB, not only

correlates to an increase in the lifetime of these lesions, but a decrease in the probability of faithful repair and therefore increased biological effectiveness [18-20].

It is important to note that biological effectiveness for a range of biological end-points also increases with decreasing photon/electron energy due to a corresponding increase in the average LET of the electrons produced [21-24]. As a result, calculating the RBE, using high energy γ -rays as the reference radiation is likely to give a higher RBE than if an orthovoltage x-rays were used, with the RBE also dependent on filtration used. The fast electrons produce a cascade of lower energy electrons as they slow down. As a result a significant proportion of energy is deposited as low-energy electron track-ends (0.1 – 5 keV), increasing from ~ 33% for ^{60}Co γ -rays to 49% for 220 kV x-rays [12]. Studies using ultrasoft x-rays (review by [22-26]) have demonstrated significantly enhanced RBEs for these low energy electrons. RBEs of ~3 are observed for low energy (0.3 keV), very short electron tracks (<7 nm) due to close proximity of the resulting ionization (LET ~40 keV/ μm). These densely ionising short electron-track ends are believed to be the biological relevant component of all low-LET radiation and the overall variation with photon energy resulting from the variation in their relative contribution to total dose [27].

It is often stated that DNA damage from low-LET radiation is a result of the indirect effect, while for high-LET it is produced by the direct effect. However both the direct and indirect effects play a role in both cases and the resulting DNA damage cluster will often consist of both direct and indirect damage. The contribution of indirect damage can be reduced by scavenging the hydroxyl radicals or performing the irradiations under hypoxia. For low-LET radiation this can have a large effect due to the small number of lesions making up a cluster,

with many of the potential DSB not being formed (if both strand breaks are produced by the indirect effect) or will be reduced to a SSB (if one of the strand breaks is a result of the direct effect). However the clustered damage produced by high-LET radiation will typically contain many more lesions produced by both the direct and indirect effect, where removal of indirect damage is less likely to prevent DSB formation. So even for high-LET radiation the indirect effect contributes to the complexity and therefore repairability of the DNA damage.

The biological effectiveness of radiation is critically dependent on the concentration of oxygen at the time of irradiation [28]. Cells and tissues irradiated under hypoxic conditions are significantly less sensitive to radiation than when oxygen is present, with the OER varying most between 0.05% and 2.5% O₂. The OER for low-LET radiation is typically between 2.5 and 3.5, but will vary slightly depending on the biological system and end-point. For high-LET radiation, the OER falls with increasing LET, approaching a value of 1 at an LET of ~ 200 keV/μm. The mechanism responsible for this effect is generally known as the oxygen-fixation hypothesis. Following irradiation, indirect damage will be produced in DNA predominately via hydroxyl radicals ($\cdot\text{OH}$) ultimately producing a DNA radical (DNA \cdot). If oxygen is present then this radical can react to produce a non-restorable organic peroxide (DNA-O₂ \cdot) which subsequently reacts to produce DNA-OOH. This chemically 'fixes' the damage and repair now requires enzymatic processing via a biological DNA repair pathway. However in the absence of oxygen, the DNA radical can be restored to its reduced form in the presence of a reducing species, such as thiols, with a sulfhydryl (SH) group enabling restoration of the original undamaged DNA structure. For low-LET radiation, sites of DNA damage will be dominated by relatively simple clustered lesions. As a result, the probability of repairing these lesions can be easily modified by reducing the complexity of the lesion by removing oxygen. While for

high-LET radiation, even in the absence of oxygen complex lesions will be formed which are difficult to repair, so modulating the level of oxygen at the time of irradiation will have little effect.

IR is also known to efficiently produce non-DSB clustered lesions, consisting of two or more lesions either on the same or opposite strands but not including a DSB. For x-rays they are produced with a frequency of approximately 4 – 8 times greater than DSB [29], with the frequency and spectrum dependent on radiation quality [30]. The presence of neighbouring damage sites significantly impairs repair [29, 31, 32] which increases the probability of them being present during replication, resulting in replication-induced breaks/DSB from stalled replication fork and enhanced mutation frequencies [33-35].

DNA fragments and chromosome aberrations

The packing of DNA in the nucleus can also play a role in determining biological response. DNA is wrapped around histones forming nucleosomes (~10 nm), which fold up to form 30 nm chromatin fibre, which forms loops ~ 300 nm in length. These fibres are then folded to produce a 250 nm-wide fibre coiled into the chromatid of a chromosome. The individual chromosomes occupy distinct regions called chromosomes territories within the nucleus [36]. Since high-LET particles deposit energy along discrete densely ionising tracks, they can produce spatially correlated DSB across these higher orders of DNA packing (see figure 3) which has been demonstrated theoretically and experimentally [37, 38]. These show an enhancement in DNA fragments of < 300 kbp, with a peak at ~80 bp. These can result in the yield of DSB being underestimated due to the limited resolution of commonly used experimental assays [39]. Also the close proximity of the DSB (including complex DSB) may

also result in illegitimate repair between these sites. Experiments characterising and sequencing high-LET α -particle induced HPRT mutation have shown that some of the mutations were more complicated than simple deletion events [40, 41].

If DNA DSB fail to rejoin, they may result in the loss of DNA at the end of a chromosome, which would result in a terminal deletion. Alternatively broken ends may result in miss-repair as a result of rejoining with free ends of surrounding radiation induced DSB. The probability that this may occur is therefore dependent on the proximity of surrounding DSB. Large-scale DNA deletions and rearrangements can be observed as chromosome aberrations in metaphase. Generally if asymmetric aberrations are formed (e.g. dicentric + acentric fragment or rings) this may impede separation at metaphase and ultimately result in cell death via mitotic catastrophe. Alternatively cells may subsequently die due to significant loss of genetic material within the acentric fragment (forming a micronuclei), while rearrangements resulting in symmetrical reciprocal translocations or small deletions often enable cells to survive and continue to divide. At low doses the frequency of aberrations typically increases linearly with dose, with rearrangements occurring between DSB produced by a single track, while at higher doses the two DSB undergoing pairwise interaction are more likely to result from independent tracks resulting in a significant quadratic (D^2) component to the dose response due to increasing DSB density. While it is often assumed that the DSB forms two free ends which may separate and incorrectly rejoin with other free ends (breakage-and-reunion mechanism); the Revell's exchange theory proposes that unstable lesions are formed, which do not separate unless they interact with a second unstable lesion and initiate an exchange [42]. It has also been proposed that in addition to pairwise interactions a single radiation induced lesion may interact and initiate an exchange with undamaged DNA.

Radiation track structure and associated pattern of energy deposition for different radiation qualities also varies significantly on the nuclear/micrometre scale. For example, 1 Gy of γ -rays typically correspond to ~ 1000 independent electron tracks traversing and interacting with a typical mammalian cell nucleus, producing ~ 40 DSB (table 1) relatively homogeneously distributed. While the same dose of high-LET α -particles corresponds to only a few tracks traversing the nucleus. This results in a heterogeneous distribution of correlated DSB along the particle track, both within and between traversed chromosome territories. The introduction of multiplex fluorescent in situ hybridisation (mFISH) and related cytogenetic techniques capable of labelling all the chromosomes in the genome was key in revealing the extent of rearrangements that were actually taking place. At low doses, x-ray irradiated lymphocytes mainly induced simple chromosomal rearrangements (maximum of two breaks in two chromosomes), while high-LET α -particle exposure corresponding to an average of one track per nucleus, were found to produce predominantly complex aberrations (exchanges involve 3 or more breaks in 2 or more chromosomes), as illustrated in figure 4. These included a wide spectrum of aberrations, with an average complex involving six breaks in four chromosomes [43, 44], with the number of chromosomes traversed correlating with the expected number of territories traversed. Additionally, the ratio of complex-to-simple aberrations was found to increase with increasing LET of the α -particles (for a mean of 1 alpha per cell) [45]. This relates to the increase in the number and complexity of DSBs along the track. The importance of the relative distribution of DSB across the nucleus has also been demonstrated by modifying the distribution of protons using a proton microbeam [46].

However it is likely we are still not seeing the true extent of chromosomal rearrangements using these techniques. For example, intra-chromosomal rearrangements (such as an inversion) will generally be missed, as well as rearrangements involving fragments smaller than ~ 10 Mbp corresponding to the resolution of the technique. This missing information could also provide the key to explaining the formation of some of the complex exchanges observed which appear to require three or more break points all coming together at the same time [47].

Dose rate

Studies using pulse field gel electrophoresis (PFGE) clearly demonstrate that the majority of low-LET induced DSB are quickly repaired with a half time of ~ 20 min, with most DSBs being repaired after 1 hour (which is shorter than the kinetics observed using γ H2AX foci) [48]. Complex DSB will generally repair at a slower rate. As a result, at clinical dose rates (~ 2 Gy/min) it can be assumed that repair only starts following irradiation, with all the DSB present and potentially available for pairwise interaction to produce chromosome rearrangements. However when the dose rate is reduced, such that some of the DSB will be repaired during the irradiation, this leads to a reduction in the number of breaks present at any one time and therefore their density, so reducing the biological effect. The dose response curve will eventually become linear when the dose rate is low enough (~ 0.1 Gy/h) that pairwise interactions between DSB in different tracks become negligible, with no further sparing if the dose rate is reduced further [49]. However, at longer irradiation times the response may be modified as a result of redistribution of cells through the cells cycle and repopulation. These effects play an important role in radiotherapy and led to the concept of sublethal damage (SLD) which will repair unless it interacts with another site of SLD to form

lethal damage. If an exposure is fractionated, or delivered at low dose rate, then the number of SLD present at any one time will be reduced, decreasing the probability of interaction and therefore biological effect for a given dose. Accurately accounting for the change in biological effect with dose rate is particularly important with respect to brachytherapy where for example irradiations may vary between dose rates of the order of 0.5 Gy per hour delivered over 1 to 4 days, to high dose rate delivered in 3 to 12 fractions.

While it was commonly assumed that increasing the dose rate above the conventional dose rates would not have any effect, recent *in vivo* experiments have demonstrated that this might not be the case at ultra-high dose rates (>40 Gy/s) [50-52]. This has been termed “FLASH” radiotherapy and remarkably has been demonstrated to spare normal tissue, while maintaining tumour control level and therefore could result in a substantial enhancement in the therapeutic window [53, 54]. While the underlying mechanism is still to be identified, oxygen depletion has been proposed as a potential mechanism, resulting in transient hypoxia and there is preliminary data that indicates changing the oxygen status of the tissue does modulate the effect.

Track structure modelling

For proton therapy a constant RBE of 1.1 is assumed clinically, although there is increasing evidence which suggests that this is sub-optimal. Many studies show that the RBE significantly increases in the Spread-Out-Bragg-Peak (SOBP) distal region and beyond due to increasing LET as the protons slow down (e.g. [55-58]). While for carbon ion therapy, taking into account the variation in RBE with LET is an integral part of the treatment planning process typically using phenomenological approaches, most notably the local effect model [59, 60] and the

microdosimetric kinetic model [61, 62]. However in order to explore the underlying biological mechanisms there is also a need to develop multi-scale models to link the physics, chemistry and biology.

The original pioneering work on modelling radiation track structure, the diffusion controlled reaction of the radicals and resulting DNA damage complexity (e.g. [17, 63, 64]), along with their subsequent extension to radiotherapy light ions [65, 66] was achieved by writing bespoke Monte Carlo code. More recently there has been an explosion of work in this area due to development of extensions to widely used, publically available Monte Carlo platforms (e.g. Geant4-DNA [67, 68] and TOPAS-nBio [69]).

While some studies determine the variation in the RBE for DSB induction with radiation quality, this does not tell the whole story or necessarily correlate with other biological endpoints such as cell survival. It is important that the complexity of damage is also taken into consideration, which will require the modelling of both direct and indirect damage. Additionally the fate of the cell will ultimately be critically dependent on the response of the cell as it attempts to repair the damage and current research involves developing models to investigate the differential response of known DNA repair pathways, with radiation quality (e.g. [70-73]). However, currently these models generally have limited consideration to the environment of the nucleus, such as the presence of oxygen, other reactive molecules (such as thiols), scavengers along with subsequent signalling and reactions. These are likely to be important considerations when trying to model effects, such as FLASH.

It is also important to model the genomic rearrangement resulting from miss-repair of pairwise interactions between sites of DNA damage and how these vary with dose, dose rate and radiation quality [74]. These have resulted in the expansion of Monte Carlo track structure codes to include detailed physical models of chromosome packing within the cells with a particular nuclear geometry [67, 69, 75-77]. This approach not only allows the correlation of DSB along the tracks to be taken into account but can include different repair kinetics for simple and complex DSB.

Summary

IR is unique in that it deposits energy along highly structured tracks of ionization and excitation events, with the spatial and temporal distribution of these events ultimately driving the subsequent biological response. It not only explains why IR is so effective but also the variation in RBE for different qualities of radiation. This is a multifaceted issue with the spatial distribution important on the nanometre scale, the micrometer scale and for tissue the millimeter scale (figure 3).

IR is uniquely very efficient at producing clustered DNA lesions. While it is common in the literature to just discuss DSB as the critical lesion, this disguises the fact that IR will also produce complex DSB, with both the quantity and complexity of these complex DSB typically increasing with LET. The increased complexity not only decreases the rate of repair, but also the probability of miss-repair, and complex DSB are therefore more biologically effective than simple DSB. While for low-LET radiation, induced DSB will typically be predominantly produced randomly across the nucleus, high-LET particles will produce multiple DSB along the path of the particle, increasing the chance of producing correlated DSB across nucleosomes,

chromatin as well as within a chromosome and between neighbouring chromosome territories (figure 3). The close proximity of these DSB increases the probability of miss-repair through pairwise interactions, which can ultimately result in complex gene mutations and complex chromosomal rearrangements. As a result the yield and pattern of genetic rearrangements occurring following irradiation is not only dependent on dose but also radiation quality.

Understanding the mechanisms underlying the biological effectiveness of IR can provide an important insight into ways of increasing the efficacy of radiotherapy, as well as the risks associated with exposure. A multi-scale approach is needed for modeling, not only considering the physics of the track structure from millimeter scale down to the nanometer scale, but also the structural packing of the DNA within the nucleus, the resulting chemistry of the resulting highly reactive reducing (e^-_{aq} and H) and oxidizing species ($\bullet OH$) in the context of the highly reactive environment within the nucleus, along with the subsequent biological response.

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Table 1. Approximate number of DNA lesions per cell produced following a dose of 1 Gy of low-LET photons [13].

DNA Lesion	Number per Gy
Double-strand breaks (DSB)	40
Single-strand breaks (SSB)	1,000
Base damage	>2,000
DNA-DNA crosslinks	30

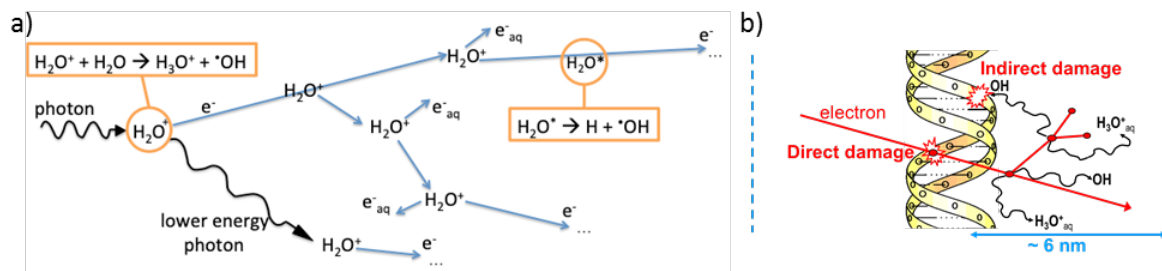


Figure 1. Illustration of interactions with water and associated DNA damage. a) an x-ray undergoing a Compton interaction within the cell, producing a fast electron and a lower energy photon. The resulting electrons subsequently lose energy via a series of excitation (H_2O^*) and ionisation ($\text{H}_2\text{O}^+ + \text{e}^-$) events predominately with water, producing secondary, tertiary etc. electrons as they to interact and slow down (“...” represents the electron producing further interactions). Ultimately the electrons thermalize and form hydrated electrons (e_{aq}^-), while the ionised water molecules will react with the surrounding water to produce a hydronium ion (H_3O^+) and a hydroxyl radical ($\cdot\text{OH}$). Additionally some of the excited water molecules will dissociate to yield hydrogen atoms and hydroxyl radicals. The initial distribution of interaction events and resulting chemical species is not random in space and time but determined by the track structure which will also influence the subsequent diffusion controlled reactions. b) Schematic of a DNA doubles stand break being produced by a combination of direct and indirect action of ionising radiation. Radiation can either produce damage by interacting through direct ionisation or excitation of the DNA molecule, but also indirectly as a result of a reaction with free radicals, most notably hydroxyl radicals, which are produced as a result of interaction with radiation in the surrounding water. Due to the highly reactive environment within the nucleus, the diffusion distance is limited to ~ 6 to 9 nm. In addition to strand breaks shown here, base damage can also be induced as a result of direct and indirect action.

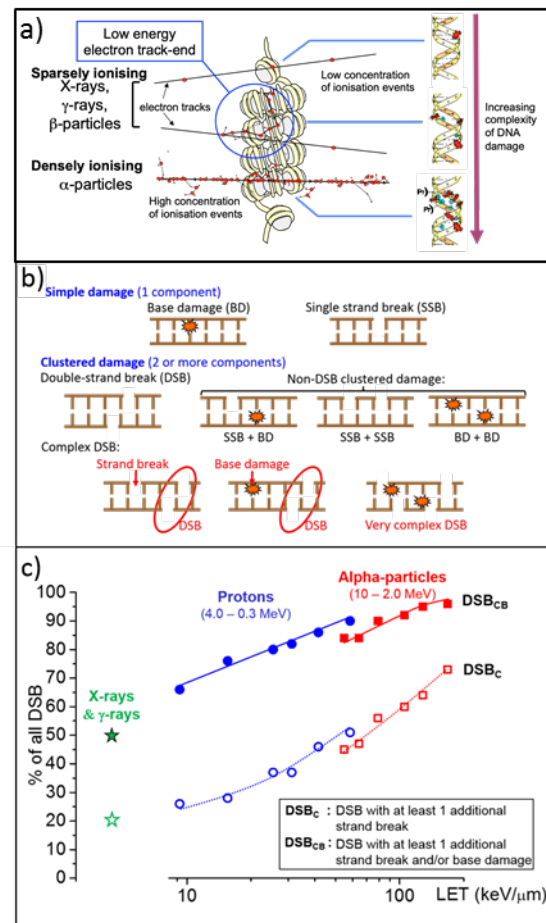


Figure 2. Ionising radiation can produce a wide spectrum of DNA damage, with frequency and complexity dependent on radiation quality. a) Schematic of low and high-LET radiation tracks (adapted from Goodhead et al [18]). Increasing ionisation density (LET) results in an increase in probability of clustered damage and increased complexity of this damage. While isolated events from sparsely ionising radiation typically result in simple isolated DNA damage, ~30 – 50% if energy is deposited as low energy (0.1 - 5 keV) track-end electrons, producing a localised increase in ionisation density. b) Illustration of the type of damage that can be produced, this not only includes simple damages such as base damage or single-strand breaks, but combinations of these. As well as DNA double-strand breaks (DSB) and complex DSB, ionising radiation can also produce non-DSB clusters of damaged bases and/or strand breaks. c) Variation in the percentage of DSB that are complex, by virtue of at least one additional strand break (DSB_C) or one additional strand and/or base damage (DSB_{CB}) with LET [16, 17] (adapted from Nikjoo et al [17] © 2019 Radiation Research Society).

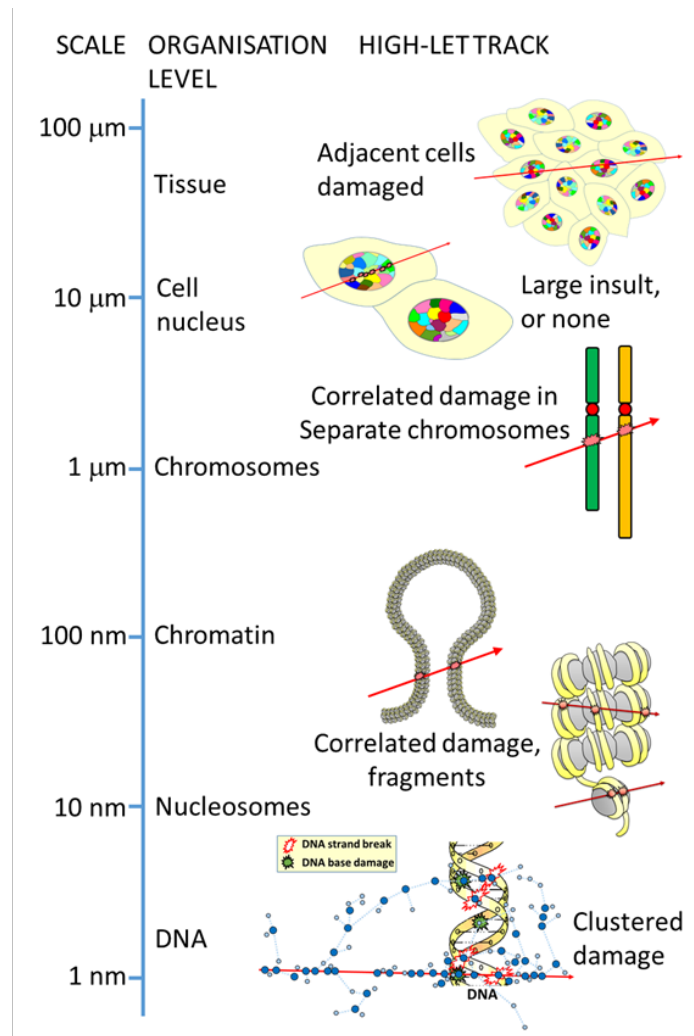


Figure 3. The pattern of energy deposition, DNA damage and in particular the correlation of these events is dependent on radiation track structure from nanometres to millimetres and plays a critical role in determining biological response. The figure illustrates this for high-LET tracks, but the tracks structures and associated damage from low-LET radiation will be very different across all these levels. Reproduced from “Goodhead DT, Mechanisms for the biological effectiveness of high-LET radiations. *J Radiat Res* 1999, 40:S1–13” by permission of the Oxford University Press on behalf of The Japan Radiation Research Society (JRRS), and the Japanese Society for Radiation Oncology (JASTRO).

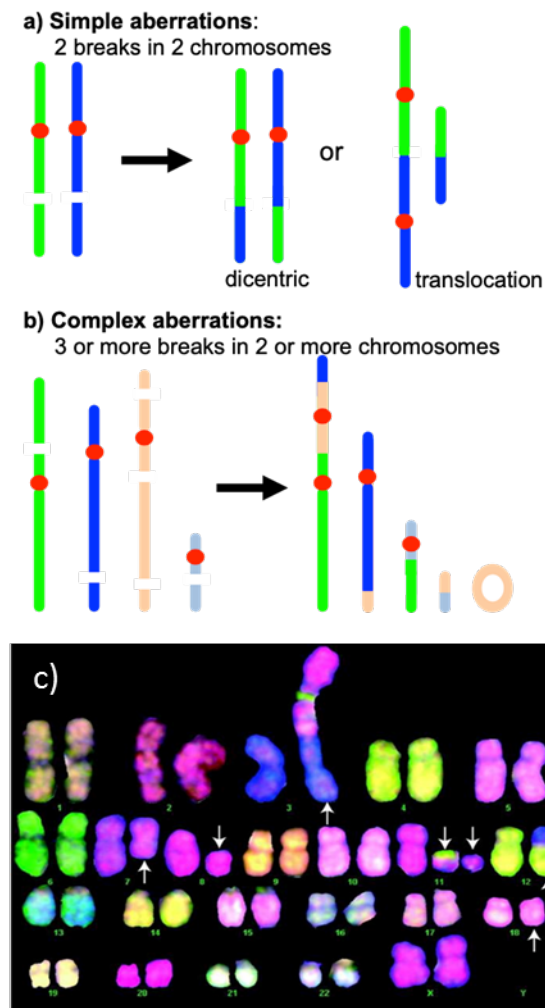


Figure 4. Examples of a) simple chromosome aberrations (defined as arising from a maximum of 2 breaks in 2 chromosomes); b) complex aberration (defined as 3 or more breaks in 2 or more chromosomes) formed by 6 breaks in 4 chromosomes; c) An mFISH karyotype illustrating an extreme aberration with 12 breaks in 6 chromosomes formed following α -particle exposure at a dose corresponding to an average of 1 track per cell nucleus (Figure adapted from Anderson et al [43], Copyright (2002) National Academy of Sciences).