Cytogenetic biomarkers of radiation exposure

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Abstract

Biological monitoring of radiation exposure relies heavily on the quantification of chromosome aberrations such as dicentrics and reciprocal translocations in the peripheral blood lymphocytes of exposed and potentially exposed subjects. The differences in the spatial deposition of energy and the quality of damage initially induced between individual low and high-linear energy transfer (LET) radiation tracks are known to impact dramatically on the type and complexity of chromosome aberration induced. Over the years, researchers have proposed numerous cytogenetic markers and signatures based on these differences with the aim of biologically discriminating exposure to radiation of varying qualities. Complex chromosome aberrations are a broad classification of aberration types that are known to be characteristically induced after low doses of high-LET. The mechanistic basis for complex aberration formation and the potential applicability of these complex aberration products as LET-specific biomarkers are considered.

Keywords

Introduction

The potential health risks of exposure to ionising radiation can be estimated from individualised biological assessments of dose. Some prior knowledge as to the radiation quality and conditions of exposure are necessary if these estimates are to be robust, as biological effect is influenced by linear energy transfer (LET) and dose-rate in addition to dose. In unknown or mixed dose exposure scenarios this may not always be the case. For instance, uncertainties in estimating cancer risks from the neutron component for A-bomb survivors, the potential for internal radionuclide contamination for nuclear workers and from nuclear fallout and, in assessing risks for medical workers exposed to radioactive isotopes [1, 2]. Expressed in units of keV/μm, LET specifies the average energy transferred per unit length of the track and correspondingly differentiates sparsely (e.g. x-rays, γ-rays) from densely (e.g. α-particles, neutrons) ionising radiations as low and high-LET radiation respectively. Medical radiological workers represent an increasingly large population who are occupationally exposed to varying low doses of high-LET exposures over the course of their working lives, potentially combined with low-LET radiation and chemical clastogens [3]. The risks for adverse health from such low dose chronic exposures however remains a matter of debate. Accordingly, there is a need to identify and monitor those who have been exposed to different qualities (LET) of radiation. This is also relevant for the growing number of patients receiving repeated low dose diagnostic radiation exposures of varying quality.

Current biological monitoring relies heavily on the quantification of chromosome aberrations which are detected using various means in the peripheral blood lymphocytes (PBL) of exposed subjects and include dicentrics, reciprocal translocations, micronuclei and sister chromatic exchanges [4]. Indeed, the induction of simple dicentrics and reciprocal

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translocations by low-LET radiation and their application as bio-dosimeters are very well characterized [5, 6]. The purpose of this article is to provide a general overview of radiation-induced chromosome aberrations with a focus on the types most commonly seen after exposure to high-LET radiation, to examine potential mechanisms of formation and their applicability as LET-specific biomarkers.

Dicentrics and reciprocal translocations are the 'gold' standard for bio-dosimetry

Ionising radiation is extremely effective in inducing structural chromosome aberrations. The mechanism for induction is not fully understood, but is generally accepted to conform to a model of 'breakage-first' followed by 'reunion' [7, 8]. According to this model, radiation produces a 'break' that results in the complete severance of the chromosome backbone resulting in 'ends' that are free to move. Based on pair-wise interactions, these 'ends' then have three different fates: 'restitution' to produce a cytologically normal chromosome, 'remain open' and be observed as a broken chromosome or to 'illegitimately re-join' with other broken ends and produce visible structural aberrations. At the time of proposing this model, the structure of DNA and the tertiary organisation of chromatin was not known, yet it remains the most widely held theory of chromosome aberration formation today. DNA double strand breaks (DSB) of varying complexity are an important class of damage induced after exposure to ionising radiation and are considered to be the critical lesion in this process [9-11]. In effect, radiation-induced DSB represent 'break ends' and chromosome exchanges are one consequence of the cell's attempts to repair this damage.

Structural chromosome-type aberrations can be broadly classified into two different groups. The symmetrical (or stable) types and the asymmetrical (or unstable) types, with stability

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being defined as the ability of the rearranged chromosome to proceed through mitosis with no loss (or gain) of chromosome material. Stable structural chromosome aberrations include reciprocal translocations and insertions or inversions that result in monocentric rearranged chromosomes, while unstable aberrations include dicentric chromosomes, fragments and rings (Figure 1). Studies have shown that although the chance of forming a reciprocal translocation is equal to the formation of a dicentric plus acentric fragment [12], there is a bias for the formation of acentric rings (intra-arm event) compared to centric rings (inter-arm event) [13-16]. In other words, the mechanism of exchange between chromosomes is the same and the distribution of the centromere within the exchange is random, but the proximity of breaks relative to each other will influence their chance of repair [17].
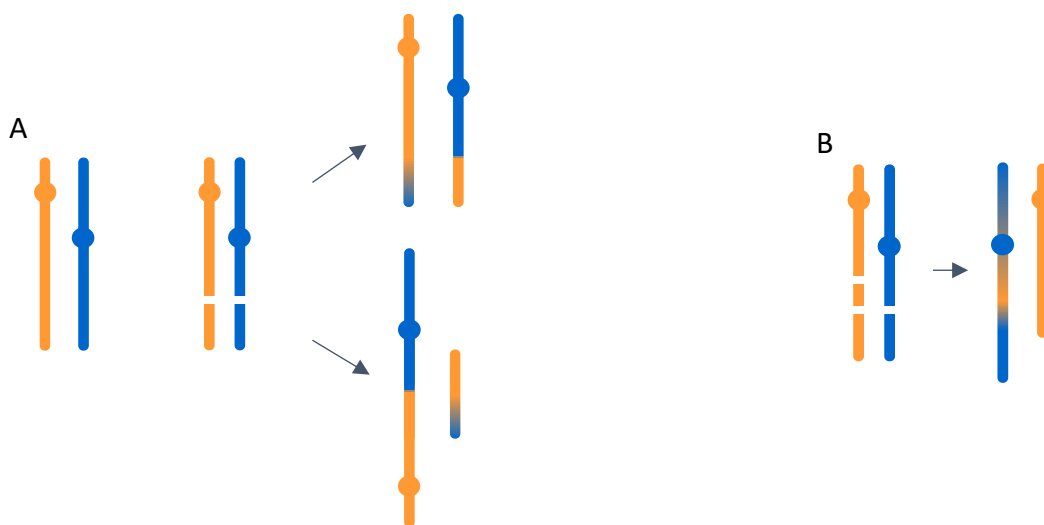

Figure 1: Cartoons depicting structural exchange formation. (A) Two breaks in two chromosomes can 'restitute' to form cytologically normal chromosomes or, incorrectly repair as a symmetrical reciprocal translocation (upper) or asymmetrical dicentric chromosome with associated acentric fragment (lower). (B) Insertions can arise when multiple breaks producing cytologically visible (~10Mb) sections of chromatin 'rejoin' with one or more than one different chromosomes.

The aberration of choice for bio-dosimetry has historically been unstable types because of the ease of visualisation using basic solid-staining techniques. Dicentric chromosomes occur at a very low level in un-irradiated persons (<0.001) [4] and increase in a linear [18] or linear-quadratic manner [19] after exposure to high or low-LET radiation respectively. Thus, estimates of whole-body dose can be determined from the PBL dicentric frequency [20]. Due to their unstable nature, the initially-induced dicentric frequency will reduce with time after exposure with a half-life of ~2-3 years [21] meaning dicentrics are useful for recent exposures only. In-homogeneity in exposure, rate and different qualities of radiation can also introduce errors in dose estimations.

In situ hybridisation (ISH) was first developed in 1969 by Gall and Pardue [22]. Over the years ISH has been revolutionised through (1) improvements in 'fixation' techniques to maintain the tertiary structure of metaphase chromosomes, (2) the ability to 'sort' individual chromosomes for efficient labelling with multiple, spectrally distinct, fluorophores [23-25] and, (3) the advancement in fluorescence microscopy and image processing software. Fluorescence ISH (FISH) whole chromosome labelling has progressed from 'painting' individual chromosomes the same colour [26], to painting two or three different chromosomes the same or different colours, to painting all the human chromosomes different colours [27, 28]. Stable reciprocal translocations can now be detected with efficiency after exposures of ~50-100mGy or greater and correlated with radiation exposure in the distant past [29-32]. Provided these aberrations are not accompanied by additional unstable elements in the damaged cell, then reciprocal translocations will persist with a half-life of ~ 6 years for the life-time of the mature PBL, after which time the observed frequency will reflect the frequency of occurrence in precursor haemopoietic stem cells [33]. Thus, FISH-based quantification of reciprocal translocations is currently the 'gold'

standard for retrospective dosimetry [5]. Reciprocal translocations are not exclusively

induced by exposure to radiation however and are accumulated throughout the human life-

time [34-36]. Accordingly, the lowest cumulative dose detectable above this background is

strongly influenced by age and inter-individual variation within human populations.

Estimates show this to increase linearly at a rate of 1.8mGy/year and 15.9mGy/year from

20-69 years for acute and chronic exposures respectively [37].

High-LET radiation efficiently induces complex chromosome exchanges

Although widely observed in solid tumours [38], complex chromosome aberrations were,

until the advent of FISH, regarded as rare events after exposure to ionising radiation and not

considered relevant in the assessment of radiobiological effects. Defined as any exchange

that involves three or more breaks in two or more chromosomes [39, 40], complex

aberration induction is now established as being strongly dependent on dose in the low-LET

range [41, 42] but largely independent of dose when the LET is >100 keV/μm. This has been

shown for α-particles [43-47], neutrons [48-50] and heavy ions [51-53]. Further, as the LET

increases from ~100 - >1000 keV/μm, the frequency and complexity of the complex

aberrations induced, also increases [45]. In addition to this, certain rearrangements that had

previously been classified as simple reciprocal translocations or dicentrics (maximum of two

breaks in two chromosomes) [40] have since been shown to be 'hidden' complex

aberrations [39, 54, 55]. As the number of chromosomes capable of being differentially

painted by FISH increased to that achieved with multiplex-FISH (M-FISH) or spectral

karyotyping (SKY) (24-colour karyotyping), then the effectiveness of detecting additional

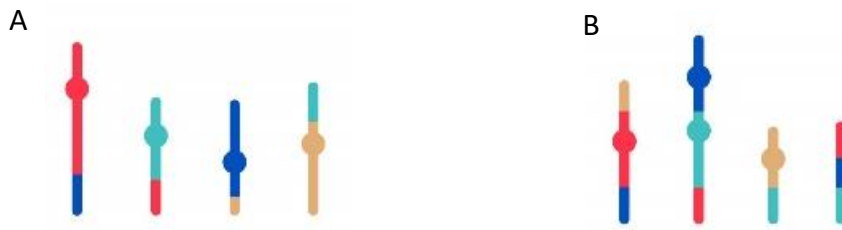complexity has also increased [56, 57] (Figure 2).

Figure 2: Cartoons depicting (A) stable transmissible complex involving 4 different chromosomes which have incorrectly repaired in a non-reciprocal manner to form 4 rearranged monocentric chromosomes and (B) non-transmissible complex involving 4 different chromosomes which have rearranged in a manner that both forms three visible insertions, dicentric and acetric elements.

Aberration complexity is not just a function of LET but is intimately associated with the qualitative structure of the radiation track [58-60]. Densely ionising radiation such as high-LET α-particles induce clustered complex DSB along the whole length of the particle track, in contrast to low-LET radiations which produce much more spatially distributed damage [61-65]. Furthermore, the range and dimensions of for example, a 5 MeV α-particle typically has limited penetrance (40 μm) and little maximum radial spread (~0.1 μm), with ~90% of energy deposited within 10 nm of the particle's path. Consequently, a high-LET α-particle is only capable of intersecting with a very small fraction of the total cell volume, which if by chance is intersected, will almost never be intersected by another track even for very high exposures [66]. Yet, α-particles and other types of densely ionizing radiation, have been shown *in vitro* to be very efficient in inducing high frequencies of complex aberrations that commonly involve three, but can be up to seven, different chromosomes [56, 67], in addition to intra-chromosomal events [68-70].

To explain how such aberrations may be formed, a model was proposed based on theoretical 're-joining' cycles [12, 54, 56] whereby complex exchanges arise through the sequential linking of smaller, independent exchanges [71] inferring that aberrations of increasing complexity are cumulative products of multiple, localised rearrangements [58, 67]. This implies that aberration complexity also correlates with the number of different chromosome territories intersected by each track, meaning the geometry of the nucleus influences the aberration type induced. Indeed a reduced frequency and a reduced complexity of complex exchanges are observed in ellipsoid cells compared to spherical cells [72, 73] with variations depending upon the angle of particle trajectory through ellipsoid cells [74].

Therefore at low dose, LET and track structure dictate the spatial and temporal proximity of initially-induced lesions, on a DNA, chromatin and nucleus-wide basis which, in turn, influences the aberration type or 'pattern' most likely to be resolved in the following metaphase [60].

Chromosome insertions as stable indicators of high-LET radiation

An important class of complex aberration is the chromosomal insertion (Figures 1-3). Mechanistically, insertions are characteristic of low doses of high- (but not low-) LET radiation due the proximity of lesions induced [44, 45, 49, 50, 57]. Further, insertions are commonly regarded as stable complex events since they can be defined as the cytologically-visible (>10Mb) product of an interstitial deletion and its subsequent 'insertion' into a different chromosome or chromosome region. Accordingly, insertions have been proposed as stable indicators of past exposure to high-LET radiation [43, 44, 49, 75]. Similarly, Brenner

and colleagues argue that large (>6MB) stable intra-chromosomal (inversions/deletions) to be preferentially induced by α-particles and neutrons citing their usefulness as long-lived LET biomarkers. However, insertions are now being resolved as being part of much larger, mostly, unstable complex rearrangements when visualised by M-FISH (Figure 2), in keeping with those previously described, meaning the potential transmissibility of these insertions is much lower than previously thought [43, 57]. This is reflected by the low relative proportion of complex aberrations which are classified as being of the transmissible-type and the low estimated frequency of damaged, but stable cells [76-78]. Accordingly, insertions are characteristic features of both stable and unstable complex aberrations induced after exposure to high-LET radiation.
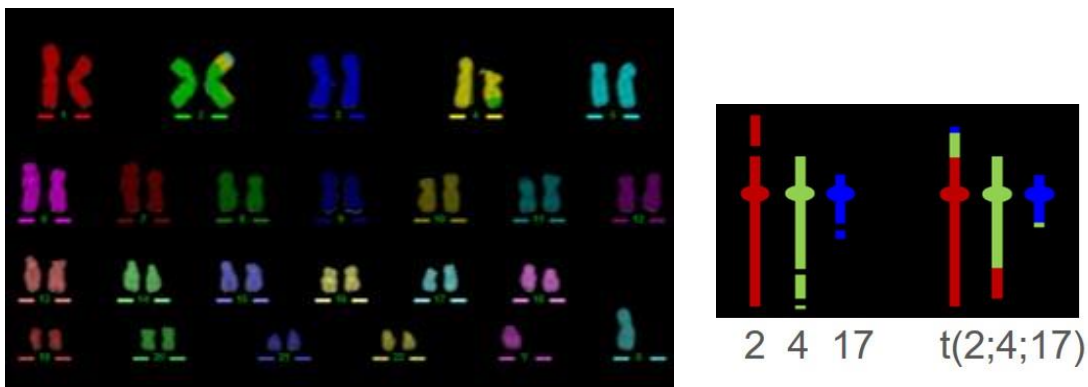

Figure 3: (A) M-FISH karyotype and (B) cartoon showing transmissible complex exchange involving chromosomes 2, 4 and 17. A section of chromosome 4 can be seen as an insertion incorrectly re-joined between the terminal end of chromosome 17 and chromosome 2.

Signatures of high-LET radiation

Various researchers have, over the years, proposed candidate 'signatures' of high-LET radiation exposure based on the differences in radiation track structure and the proximity basis of exchange formation as outlined above. The first signature to be proposed was the F-ratio [79] which predicts a low ratio of inter-chromosome to intra-chromosome exchanges, on the assumption that high-LET radiation is more likely to result in more intra-chromosomal than inter-chromosomal changes, in comparison to low-LET radiation. In a similar vein the H-ratio [15] translates to a bias for the formation of intra-arm intra-changes rather than inter-chromosomal exchanges and, the I-ratio [75] as stable inter-chromosome exchanges rather than stable intra-chromosome exchanges, after exposure to high-LET radiation. The C-ratio (complex: simple exchanges) derives from the arguments already presented in this article.

The potential application of such ratios *in vivo* is complicated however due to a number of factors including inter-individual differences in the 'background' frequency of inter-chromosome exchanges within populations [34-36, 80, 81]. Further, although complex aberrations are the predominant exchange type induced in PBL after high-LET radiation, this is not at the exclusion of simple exchanges also being formed [82-84]. A proportion of these simples will be stable reciprocal translocations, both capable of being transmitted to progeny cells [85, 86] and, being indistinguishable from those induced by low-LET radiation.

Complexity *per se* as an indicator of high-LET radiation

Complexity is the major cytogenetic feature of high-LET radiation. Differing staining and painting techniques, depending on their resolution, will reveal different elements of the total damage in each cell. The likelihood that even quite subtle (apparently) stable damage (for example an insertion or intra-chromosome exchange) [69, 70], is part of a larger complex event that will ultimately be lethal to the cell, is expected to be high, with clear implications for applicability as retrospective markers of exposure.

That said, heavily damaged cells revealed as unstable (and stable) complex aberrations, have been shown to be long lived *in vivo* many years after exposure. For instance, PBL sampled from retired workers chronically exposed to internalised plutonium (α-particle emitter) (estimated effective doses to the bone marrow in excess of 200mSv) contained significantly elevated frequencies of complex chromosome exchanges, of which ~20-50% were classified as transmissible (Figure 3) most likely resulting from dose to the red bone marrow [83]. The remaining non-transmissible (unstable) complexes are thought to represent mature T-cells which remain in their 1st interphase for months or even years after irradiation, until sampled and artificially stimulated to divide in culture. The biological reason for this long-term survival of such heavily damaged PBLs is unclear. However, similar observations, at varying frequencies, have been reported elsewhere including in plutonium workers [87-90], thorotrast patients [85, 91], veterans of nuclear testing [92], A-bomb survivors [93], astronauts and patients receiving carbon therapy [81]. Interestingly, Hande *et al* [79] found a significant correlation with estimated plutonium dose to the bone marrow (from ~500mGy) and yield of complex aberrations suggesting quantification could potentially be used for dose reconstruction. T-cell life-span and immunological status will

clearly influence the frequency of detection of any chromosome aberration, but for chronic internal exposures, complex aberrations will be induced for the biological and physical lifetime of the radionuclide. As an example, plutonium can remain in the lymph nodes for many years [94] and similar to radium-223, another α-particle emitter used in targeted radiotherapy, is deposited on the bone surface meaning both PBL and progenitor cells in the bone marrow may be 'hit' by an α-particle.

The bio-distribution of other radionuclides such as iodine-131 will vary and unlike external low-LET radiation, will result in inhomogeneity of dose which complicates assessment [95]. Further, *in vivo,* cells will be exposed to a spectrum of energies and therefore a spectrum of LETs which will influence the proportion of simple exchanges induced [84]. A linear dose response for the low yield of reciprocal translocations detected in stable cells was seen at α-particle doses of greater than 50mGy *in vitro* [96]. Curwen *et al* [75] go on to show the validity of using this to estimate α-particle dose in a small number of plutonium workers who also exhibited complex aberrations.

Overall therefore, it seems appropriate that complex chromosome aberrations *per se* should be explored further as indicators of high-LET exposure irrespective of their long-term stability and that their utility as a biomarker of high-LET exposure is used together with reciprocal translocation yields, particularly where internal contamination is known or suspected.

Conclusion

The quantification of simple reciprocal translocations or dicentrics for individual assessment of dose is well established for low-LET exposures and is being used to better understand radiobiological effects of low doses of radiation, including that received from diagnostic CT scans and by radiation medical workers [1, 2, 97-100]. For the past twenty years, researchers have sought to build on this and verify cytogenetic markers that discriminate varying qualities (LET) of radiation and through their application, identify individuals exposed to mixed or unknown exposures. All are rooted in a mechanistic understanding of the distinct differences in spatial deposition and of the quality of damage, initially induced between individual low and high-LET radiation tracks and how this relates to the complexity of chromosome aberration resolved using different techniques. The specificity of complex aberration formation after low doses of high-LET radiation coupled with the knowledge they occur rarely in the normal population, promotes complex chromosome aberrations to be essential indicators for this purpose. Their application is tempered however, by their low frequency of occurrence relative to reciprocal translocations *in vivo*, their unstable nature through cell division and by the requirement for expensive and labour-intensive techniques to fully resolve each damaged cell. Further and larger studies exploring the patterns of complexity and the frequencies of occurrence in different high-LET exposed population groups is necessary therefore before we can fully assess the practical usefulness of complex chromosome aberrations as biomarkers of high-LET exposure.

Structural chromosome aberrations as biomarkers of exposure have been discussed. Chromosome aberrations are also key biomarkers of radiation sensitivity [100] and, are proposed as important indicators of cancer risk [101, 102]. Accordingly, the detailed

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quantification of the entire chromosome aberration burden can provide information on dose and disease risk for each individual (which may alter over time), providing more insight on variations in response and outcome within populations.

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