



Overview

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 dicentric 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.

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Key words: Chromosomal biomarker; Chromosomal signature; Complex chromosome aberrations; High-LET; Radiation exposure

Statement of Search Strategies Used and Sources of Information

The key words ‘radiation exposure’, ‘chromosomal biomarker’, ‘chromosomal signature’ and ‘complex chromosome aberrations’ were used to search Web of Science and Scopus for peer-reviewed articles.

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 example, 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, remain 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

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lymphocytes (PBL) of exposed subjects and include dicentrics, reciprocal translocations, micronuclei and sister chromatic exchanges [4]. Indeed, the induction of simple dicentrics and reciprocal translocations by low-LET radiation and their application as bio-dosimeters are very well characterised [5,6]. The purpose of this review 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 pairwise 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 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, whereas unstable aberrations include dicentric chromosomes, fragments and rings (Figure 1). Studies have shown that although the chance of forming a dicentric plus acentric fragment 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 with 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].

The aberration of choice for bio-dosimetry has historically been unstable types because of the ease of

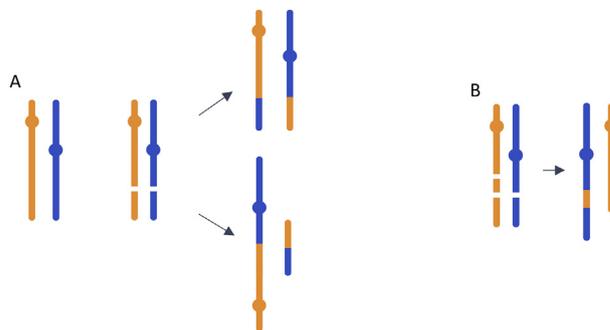


Fig 1. Structural exchange formation. (A) Two breaks in two chromosomes can ‘restitute’ to form cytologically normal chromosomes or incorrectly repair as a symmetrical reciprocal translocation (upper) or asymmetrical dicentric chromosome with associated acentric fragment (lower). (B) Insertions can arise when multiple breaks producing cytologically visible (about 10 Mb) sections of chromatin ‘re-join’ with one or more than one different chromosomes.

visualisation using basic solid-staining techniques. Dicentric chromosomes occur at a very low level in unirradiated 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 about 2–3 years [21], meaning dicentrics are useful for recent exposures only. Inhomogeneity 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: (i) improvements in ‘fixation’ techniques to maintain the tertiary structure of metaphase chromosomes, (ii) the ability to ‘sort’ individual chromosomes for efficient labelling with multiple, spectrally distinct, fluorophores [23–25] and (iii) 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 about 50–100 mGy 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 about 6 years for the lifetime 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 lifetime [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 1.8 mGy/year and 15.9 mGy/year from 20–69 years for acute and chronic exposures, respectively [37].

High-linear Energy Transfer 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]. Furthermore, as the LET increases from about 100 keV/ μm to more than 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 dicentric (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 or spectral karyotyping (24-colour karyotyping), then the effectiveness of detecting additional complexity has also increased [56,57] (Figure 2).

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

(about 0.1 μm), with about 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 ionising 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 that 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 with 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-linear Energy Transfer 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 to the proximity of lesions induced [44,45,49,50,57]. Furthermore, insertions are commonly regarded as stable complex events as they can be defined as the cytologically visible (>10 Mb) product of an interstitial deletion and its subsequent ‘insertion’ into a different

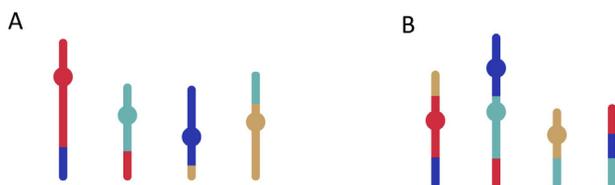


Fig 2. (A) Stable transmissible complex involving four different chromosomes that have incorrectly repaired in a non-reciprocal manner to form four rearranged monocentric chromosomes. (B) Non-transmissible complex involving four different chromosomes that have rearranged in a manner that forms three visible insertions, dicentric and acentric elements.

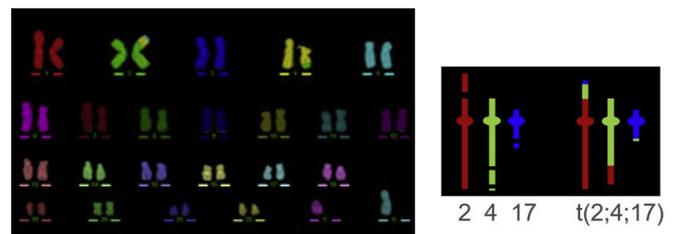


Fig 3. (A) Multiplex-fluorescence *in situ* hybridisation karyotype and (B) transmissible complex exchange involving chromosomes 2, 4 and 17. A section of chromosome 4 can be seen as an insertion incorrectly re-joined between the terminal end of chromosome 17 and chromosome 2.

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 (>6 Mb) stable intra-chromosomal (inversions/deletions) to be preferentially induced by α -particles and neutrons, citing their usefulness as long-lived LET biomarkers [70]. However, insertions are now being resolved as being part of much larger, mostly unstable complex rearrangements when visualised by multiplex-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 that are classified as being of the transmissible-type and the low estimated frequency of damaged, but stable cells [57,76,77]. Accordingly, insertions are characteristic features of both stable and unstable complex aberrations induced after exposure to high-LET radiation.

Signatures of High-linear Energy Transfer 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 [78], 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 with 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 overview.

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 [34–36,79,80]. Furthermore, 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 [81–83]. A proportion of these simples will be stable reciprocal translocations meaning they have the potential of being transmitted to progeny cells [84,85] whilst remaining cytogenetically indistinguishable from those induced by low-LET radiation.

Complexity *per se* as an Indicator of High-linear Energy Transfer 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 example, PBL sampled from retired workers chronically exposed to internalised plutonium (α -particle emitter) (estimated effective doses to the bone marrow in excess of 200 mSv) contained significantly elevated frequencies of complex chromosome exchanges, of which about 20–50% were classified as transmissible (Figure 3), probably resulting from dose to the red bone marrow [82]. The remaining non-transmissible (unstable) complexes are thought to represent mature T-cells, which remain in their first 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 [77,86–88], thorotrast patients [84,89], veterans of nuclear testing [90], A-bomb survivors [91], astronauts and patients receiving carbon therapy [80]. Interestingly, Hande *et al.* [69] found a significant correlation with estimated plutonium dose to the bone marrow (from about 500 mGy) and yield of complex aberrations, suggesting quantification could potentially be used for dose reconstruction. T-cell lifespan 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 [92] 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 biodistribution of other radionuclides, such as iodine-131, will vary and, unlike external low-LET radiation, will result in inhomogeneity of dose, which complicates assessment [93]. Furthermore, *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 [83]. A linear dose response for the low yield of reciprocal translocations detected in stable cells was seen at α -particle doses of greater than 50 mGy *in vitro* [94]. Curwen *et al.* [77] 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 dicentric 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 computed tomography scans and by radiation medical workers [1,2,95–98]. For the past 20 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 that 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 [98] and are proposed as important indicators of cancer risk [99,100]. Accordingly, the detailed 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.

Conflict of interest

The author declares no conflict of interest.

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