Nuclear 3D organization and radiosensitivity

Y A Eidelman1, S V Slanina1, A V Aleshchenko1, O V Sen’ko2, A D Kononkova3, S G Andreev1,4

1 Institute of Biochemical Physics, Russian Academy of Sciences, Kosygin str. 4, 119334, Moscow, Russia
2 Dorodnicyn Computing Centre, Russian Academy of Sciences, Vavilov st. 40, 119333 Moscow, Russia
3 National University Higher School of Economic, Kochnovsky pr. 3, 125319, Moscow, Russia
4 National Research Nuclear University MEPhI, Kashirskoye shosse 31, 115409, Moscow, Russia

E-mail: andreev_sg@mail.ru

Abstract. Current mechanisms of radiation-induced chromosomal aberration (CA) formation suggest misrepair of chromosomal lesions being in spatial proximity. In this case CAs have to depend on pattern of chromosomal contacts and on chromosome spatial organization in a cell nucleus. We were interested in whether variation of nucleus 3D organization results in difference of radiation induced CA formation frequency. Experimental data available do not provide information sufficient for definite conclusions. To have more deep insight in this issue we developed the biophysical modeling technique taking into account different levels of chromosome/nuclear organization and radiation damage of DNA and chromosomes. Computer experiments on gamma irradiation were carried out for two types of cells with different 3D organization of nuclei, preferentially peripheral and internal. CA frequencies were found to depend on spatial positioning of chromosomes within a nucleus which determines a pattern of interchromosomal contacts. For individual chromosomes this effect can be more pronounced than for genome averaged. Since significant part of aberrations, for example dicentrics, results in cell death, the proposed technique is capable of evaluating radiosensitivity of cells, both normal and cancer, with the incorporation of 3D genome information. This predictive technology allows to reduce uncertainties of prognosis of biological effects of radiation compared to phenomenological methods and may have variety of biomedical applications, in particular, in cancer radiation therapy.

1. Introduction

The term radiosensitivity is used widely as a parameter of an effect of interest. In radiobiology and in radiation therapy radiosensitivity often refers to cell death. For example, the dose of e-fold decrease in cell survival is used as a radiosensitivity parameter. In this study we use this term to characterize the sensitivity of chromosome to radiation damage which are experimentally observed as chromosomal aberrations (CAs). CAs are divided into inter- and intrachromosomal, simple and complex, etc.; the examples and definitions are presented in figure 1.
Figure 1. Pathways of induction of intra- and interchromosomal CAs. Chromosomes are schematically represented as colored sticks. Different chromosomes and/or chromosome arms are depicted in different colors. Centromeres are depicted as yellow circles.

According to the current view on radiation-induced CA formation mechanisms, aberrations are formed as a result of misrepair of chromosomal lesions being in spatial proximity [1]. Some CAs (for example, dicentrics and centric rings, see figure 1) lead to cell death, hence they can serve as a cytogenetic marker of lethal effects of radiation and as a quantitative characteristics of radiosensitivity [2, 3].

On the other hand, some CAs involving particular loci correlate with several types of malignancies [2]. The inversion between genes RET and PTC1 in chromosome 10 is associated with thyroid cancer; the translocation between genes AF4 (chromosome 4) and MLL (chromosome 11) is a predictor of acute lymphoblast leukemia; the translocation between genes ABL (chromosome 9) and BCR (chromosome 22) is a prognostic factor of chronic myeloid leukemia.

To enable CA induction, at least two lesions should be formed in chromosomal loci either being in contact at the time of irradiation (“contact first” pathway) or coming in contact shortly after irradiation (“breakage first” pathway) [1]. In both cases CAs depend heavily on chromosomal contact probability and hence on spatial organization of chromosomes in a nucleus and on contact pattern within chromosomes.

The present work is aimed at the question whether differences in nucleus 3D organization result in differences in radiation induced CA frequencies. As a marker of nucleus 3D organization, radial distribution of chromosomes in an interphase nucleus is introduced. We present the modeling approach for calculating yields of radiation induced CAs of various types taking spatial organization of a nucleus into account. This polymer physics based technique models structure of 46 human chromosomes, dynamics of megabase subunits of all chromosomes, as well as DNA double-strand break induction, repair and misrepair. CA frequencies are shown to depend on radial distribution of chromosomes. For individual chromosomes this effect can be more pronounced than for genome averaged. The approach presented demonstrates the principal opportunity of predicting radiosensitivity of both normal and malignant cells that may be beneficial for applications in tumor radiotherapy.

2. Methods

Multiscale modeling of 3D organization and dynamics of all 46 chromosomes in a human cell nucleus is performed by extension of methods developed previously [4, 5]. Each chromosome is represented as a coarse-grained chain of semi-rigid elements corresponding to megabase sized chromosomal subunits, or domains [5, 6]. Interphase chromosome structures are established in the course of mitotic chromosome decondensation. Subunits from the same chromosome interact through a Lennard-Jones potential, subunits from different chromosomes through a truncated Lennard-Jones potential with only the excluded volume component. A Metropolis method with the dynamic variant of Monte Carlo
(MC) technique is used, at each MC step positions of elements are randomly changed [4, 5]. G1 state of the nucleus is reached when gyration radii of all chromosomes cease to change. In order to make correct comparisons between our predictions and FISH data on radial distribution of chromosomes in human lymphocyte nuclei [7], we design the following scheme of modeling of chromosome location in a nucleus. The spherical nucleus with 6 μm diameter is divided into 5 concentric layers of equal volume. Chromosome center of mass is placed in one of the four layers (except of the one adjacent to the nuclear shell) with probabilities $p_1$, $p_2$, $p_3$ and $p_4$. Model 1: probabilities $p_i$ are chosen so that distribution of domains of a given chromosome in five layers agrees with the experimental distribution of FISH signal [7]. Radial distribution of chromosomal domains correlates with that for centers of mass but they are not the same: center of mass can be in one layer while part of domains in other layers, especially for large chromosomes. Model 2: probability $p_1$ equals $p_4$ for the same chromosome in model 1, $p_2$ equals $p_3$ in model 1, etc. Thus the chromosomes which are preferentially peripheral in model 1 (i.e. high $p_1$ and low $p_4$) are preferentially central in model 2 (i.e. high $p_1$ and low $p_4$) and vice versa.

Modeling of chromosomal aberration formation at the contacts of damaged subunits is performed as follows. DNA double-strand breaks (DSBs) induced by $\gamma$-rays are distributed uniformly (on average) over the genome; they are eliminated in the course of repair by non-homologous end joining pathway; rates of lesion contact formation and decay are determined on the basis of calculated dynamics of all chromosomal subunits [4, 5]; if two damaged subunits come in contact before they are eliminated, they interact (with probability $p$, free parameter) resulting in exchange aberration formation. Identification of CAs as simple or complex is done by classification used in [8] for mFISH-detected aberrations. The term “apparently simple” used hereafter means all CAs classified as simple by mFISH technique. They include real simple aberrations as well as complex aberrations misidentified as simple (e.g. if one of exchanges comprising the complex CA is an inversion not detectable by mFISH).

3. Results

Two types of cells with different spatial organization of nucleus were studied. In this work the 3D organization of nuclei is characterized by the radial distribution of chromosomes. In cells of the first type (nucleus model 1) radial distribution of chromosomes in a nucleus is modeled so that it is able to reproduce the experimental distribution measured by the FISH technique in human lymphocytes [7], see Methods. In model 1 most of big chromosomes are distributed mainly at nucleus periphery and most of small chromosomes occupy the middle of the nucleus. In cells of the second type (nucleus model 2) we introduce another, hypothetical distribution of chromosomes.

To elucidate the impact of spatial distribution of chromosomes in nuclei on formation of radiation induced chromosomal aberrations we determine how structural parameters of chromosomes, chromosomal contacts and chromosomal aberrations are shaped by spatial distribution of chromosomes in nuclear space. We build radial profiles of chromosomes in nuclei by exploring model 1, figure 2. Next, we generate another nucleus model in which, unlike nucleus of type 1, radial distribution of chromosomes is more centered, figure 3. For both nucleus types we calculate the following structural characteristics: radial distribution of chromosomal domain density, domain pairwise contact radial distribution, total number and number of intra- versus inter-chromosomal contacts, contact distribution of genomic separation between contacting subunits.

One can observe from figure 4 b that chromatin is distributed in model 1 preferentially at nuclear periphery. 72% of chromat in is located at distance more than 2 μm from the center, i.e. further than 2/3 of nucleus radius. It corresponds to relatively homogeneous chromat in density, figure 4 a, red curve. In model 2 chromatin is located generally closer to the nucleus center: only 54% of chromat in is further than 2/3 of nucleus radius (figure 4 b) and chromat in density is dramatically higher at low distances than at high distances (figure 4 a). We conclude that cell type dependence of chromat in density radial distribution is shaped by distribution of chromosomes in 3D space of cell nucleus.
Figure 2. Radial distribution of chromatin in 23 chromosome pairs in cells of first type, model 1. Simulated distribution of chromosomes in cells of first type (model 1) are compared with FISH data [7] for human lymphocyte cells. A nucleus is divided into five spherical layers of equal volume, layer 1 is central, layer 5 is adjacent to the nuclear shell.

Figure 3. Radial distribution of chromatin in 23 chromosome pairs in cells of second type, model 2. Data on chromosome distributions generated by model 1 are presented for comparison.
Figure 4. Radial distribution of chromatin in nuclei of two types. (a) - radial dependence of density of chromosomal subunits, or megabase domains in a nucleus. (b) - probability density of location of chromosome subunits in a spherical layer at distance $r$, $r+\delta r$ from nucleus center.

Difference in spatial arrangements of chromosomes for nuclei of two types results in differences in radial distributions of total, intra and inter-chromosomal contacts (figures 5, 6). Two models differ significantly in total number of interchromosomal contacts (37200 vs 47900) whereas total number of intrachromosomal contacts is almost unchanged (18200 vs 19200).

Distributions of intrachromosomal contacts over genomic distance between contacting subunits, $n(s)$, are well correlated for nuclei types 1 and 2, figure 7. Integral of $n(s)$ equals the total number of intrachromosomal non-neighbors contacts. Comparing two models, increase of total contacts by 2.6% exists in model 2 with respect to model 1 for genome averaged $n(s)$ distribution.

To predict frequency of chromosomal aberrations formed at contacts of damaged chromosomal loci it is necessary to assess the probability of contact-to-exchange formation, i.e. the probability that a contact of two damaged sites results in an exchange aberration. This parameter was determined by modeling simulation and description of experimental dose response curve for simple dicentrics induced by low LET $\gamma$-radiation in human lymphocytes [8]. These data were obtained by labeling of all chromosomes with multiple color fluorescence probes, or multicolor FISH (mFISH), figure 8.

The term complex (exchange) means a sum over all type of exchanges where number of damaged chromosome subunits is more than two. Scoring of aberrations in our in silico experiments is

Figure 5. Radial distribution of total (intra- and interchromosomal) contacts for two nucleus types. Model 1 – 55400 contacts per nucleus, model 2 – 67100 contacts.
Figure 6. Intra- and interchromosomal contacts radial distributions for two nucleus models. Contacts between neighbors are excluded. (a) – intrachromosomal. Model 1 – 18200 contacts per nucleus, model 2 – 19200 contacts. (b) – interchromosomal. Model 1 - 37200 contacts per nucleus, model 2 – 47900 contacts.

Figure 7. Distribution of intrachromosomal contacts over genomic distance between contacting subunits for two nucleus models, n(s). Distribution is genome averaged, i.e. averaged over all chromosomes. Only non-neighbor contacts are considered.

Figure 8. Dose response for γ-ray induced apparently simple dicentrics, observed by mFISH experiment on human lymphocytes [8] and by computer experiments with multilevel modeling technique developed here. Simulation for model 1, contact-to-exchange probability p=0.013. performed in concert with the procedure applied in experimental mFISH scoring [8]. One can observe from figure 9 that aberrations yield, both simple and complex, is increased for nuclei of type 2 with respect to type 1. This conclusion is in agreement with previous observations (figures 5, 6) about increased yield of contacts per nucleus type 2.

Figure 10 demonstrates the results of in silico experiments to determine the aberration frequencies, both simple and complex, for nuclei with different spatial organization. These data suggest that in nuclei with more centric chromatin (model 2) there are more aberrations than in nuclei with more peripheral chromosome distribution (model 1). The frequency difference depends on type of aberrations, and in general complex aberrations are more sensitive to chromosome distribution than simple aberrations (the same is seen for the dose curves, see figure 9). Therefore, nucleus model 2
Figure 9. Dose response curves for $\gamma$-ray induced chromosomal aberrations predicted for nuclei differing in spatial organization. $p=0.013$. (a) - apparently simple dicentrics; (b) - visible complexes.

Figure 10. Predicted frequencies of simple and complex aberrations induced by $\gamma$-rays at dose 5 Gy in two cell types with different spatial nuclear organization (model 1, 2). (a) – simple exchange aberrations, intra- and interchromosomal. (b) – complex aberrations, intra- and interchromosomal.

where contacted domains are located preferentially in the center of nucleus, than in model 1 (figures 5, 6) is more radiosensitive in accord with the criterion of complex aberration formation.

Next, we study chromosomal aberration frequencies for specific chromosomes. We choose pairs of chromosome 4 and 11, as well as 9 and 22, because these chromosomes contain important proto-oncogenes. Their specific rearrangements, due to exchange aberrations of translocation type, may result in human malignancy with high incidence frequency. As nononcogenic reference point we selected the pair of chromosomes 4 and 7. In model 1 these chromosomes are amongst the most peripheral [7], whereas in model 2 they are located near the nucleus center. This predicts that contacts between them have to be rare for model 1 and frequent for nucleus model 2.

Assessment of radiation induced aberrations between pairs of specific chromosomes for two models reveals complex relationship between nuclear structure and exchange aberrations (figure 11). Exchange yield may be either independent (figure 11 a) or dependent (figure 11 b, c) on nuclear organization type. The highest difference is 4.9 times for exchanges between chromosomes 4 and 7.
Figure 11. Frequencies of exchanges (simple plus complex) between specific chromosomes. (a) – chromosomes 4 and 11, (b) – chromosomes 9 and 22, (c) – chromosomes 4 and 7. γ-rays, D=5 Gy.

The results in figure 11 suggest that different cell types may have very different radiosensitivity with respect to specific chromosome rearrangements while having similar radiosensitivity with respect to total aberrations yield.

4. Discussion

The general aim of this work is to assess complex relationships between nuclear organization and radiation induced chromosomal aberrations. Experimental information is highly obscure in this field. The multilevel mechanistic modeling approach presented here demonstrates a wealth of opportunities to have deeper insight in this problem.

The new information about contacts obtained in in silico experiments reveals that the shape of genome-averaged distribution of genomic separation between contacting subunits within a chromosome n(s) is almost independent of type of nucleus organization considered. The total number of contacts, excluding pairs of neighboring domains, differs between models slightly, by 2.6%. These estimates are in accord with the data in figure 6 where the total number of intrachromosomal contacts (integral on r) differs by 4% between models. On the other hand, interchromosomal contact differences between nucleus models 1 and 2 are much higher, about 30%. We propose the following explanation of this finding. Larger chromosomes give larger contribution to the yield of chromosome aberrations [4] and “surface” mechanism of exchange aberrations formation (i.e. interchromosomal exchanges are formed at the contacts of damaged subunits on the surface of chromosomal territories) operates for at least simple exchanges [4]. Larger chromosomes have larger surface area. In model 1 large chromosomes are positioned mainly near the nuclear membrane and each chromosome contacts few others. In model 2 large chromosomes are preferentially close to the center of the nucleus and they contact many other chromosomes. Small chromosomes in model 2 are mainly placed at the periphery and have few contacts relative to model 1. Since smaller chromosomes have low overall impact on total number of interchromosomal contacts, these findings reveal that interchromosomal contacts have to be more abundant in nucleus model 2 with more centric positions of larger chromosomes. However, we still cannot exclude the possible impact of another factor influencing contact differences, as to limited number of nuclei simulated (50) for each type of model, although the statistics of radiation damage simulations by Monte Carlo is 50000 per dose point per model, or 500000 in total. This question requires further investigation.

To envision three-dimensional view on nuclear organization and 3D pattern of chromosomal contacts we generated in silico the snapshot of nucleus configurations of two types, corresponding to models 1 and 2, figure 12. Multiple contacts of loci on chromosomes 9 and 22 (figure 12 b) may result in interchromosomal exchange aberrations between these chromosomes in model 2 whereas rare or no contacts (figure 12 a) do not result in exchange aberration in model 1. Since these chromosomes contain oncogenes ABL and BCR, the high accurate prediction technologies of this kind of rearrangements are requested and it will have important scientific and bio-medical significance.
We show that different spatial organization of a nucleus may underlie different frequencies of intra- and interchromosomal contacts which affect the frequency of $\gamma$-ray induced chromosomal aberrations. The idea that the problem of elucidation of chromosome aberration mechanisms primarily comes down to a problem of chromosomal contacts was proposed in [9] on the basis of polymer modeling of human chromosomes. This proposal is supported by chromosome conformation capture technique (Hi-C) measurements of intrachromosomal contacts [10]. The results of the present work further confirm the conclusion in [9] and uncover the complex aspects of the contacts vs chromosome aberrations problem. Further studies are required to quantify chromosome structure and chromosome-chromosome interactions in order to explore complex relationships between nuclear 3D organization and chromosomal aberrations.

Talking about perspectives, one can mention that the proposed in silico approach allows assessment of radiosensitivity of normal and cancer cells by incorporating differences in their nuclear 3D organization, as well as future generalization to ionizing radiation of different quality, i.e. ions of different linear energy transfer (LET). Our approach is capable to take into account variations of individual radiosensitivity which may be related, in particular, to variations of 3D genome structure. Spatial organization of a nucleus is proposed to serve as a prognostic marker of sensitivity to radiation induced cancer and as a tool of distinguishing sensitive human subpopulations. This may be of great significance in molecular epidemiology and risk prediction studies, including risks from cosmic particles [11]. These ideas are in line with the modern tendency of using radiobiological and genomic information in radiation therapy. Current studies are aimed at exploring a role and visualization of spatial distribution of complex DNA double strand breaks in human tumor therapy by carbon ions [12]. Another important task concerning our aims of research is a physical and biological determinants of radiotherapy toxicity under individual plans of irradiation [13]. Combining in vivo and in silico data on genomics, nuclear 3D structure and radiosensitivity one can hope to identify patients with reduced toxicity or improved tumor cure. Chromosomal exchange aberrations like translocations may serve as a test to identify patients with increased radiosensitivity. Detection of chromosomal translocation in blood lymphocytes was proposed to be considered as a perspective predictive assay for detection of radiosensitive individuals [14]. Our multilevel modeling approach enables additional studies of matters mentioned above in order to predict outcomes of future experiments and allows to acquire new information in the fields where conducting experiments on patients is impossible.
In conclusion, the work presented demonstrates that 3D organization of a nucleus may affect frequencies of radiation induced chromosomal aberrations of different complexity through spatial distribution of contacts and chromosome topology. The predictive mechanistic approach allows to assess radiosensitivity of normal vs cancer cells with lower uncertainties than purely phenomenological methods. The results and in silico technologies presented may promote a search of new directions for individualization of radiotherapy planning though it is obviously only beginning of the road.

Acknowledgements

This work was supported by grant 14-01-00825 from Russian Foundation for Basic Research and Competitiveness Program of National Research Nuclear University MEPhI.

References

[1] Bryant P E Origin of chromosome aberrations: mechanisms 2007 in: Chromosomal alterations: methods, results and importance in human health ed G Obe and Vijayalaxmi (Berlin Heidelberg: Springer-Verlag) pp 177–99

[2] Durante M and Loeffler J S Charged particles in radiation oncology 2010 Nat. Rev. Clin. Oncol. 7 37–43

[3] Carante M P and Ballarini F Calculating Variations in Biological Effectiveness for a 62 MeV Proton Beam 2016 Front Oncol. 676

[4] Eidelman Yu A, Ritter S, Nasonova E, Lee R, Talyzina T A and Andreev S G Prediction of dose response for radiation induced exchange aberrations taking cell cycle delays into account 2006 Radiat. Prot. Dosim. 122 185–7

[5] Eidelman Y A, Slanina S V, Salnikov I V and Andreev S G Mechanistic modelling allows to assess pathways of DNA lesion interactions underlying chromosome aberration formation 2012 Rus. J. Genet. 48 1247–56

[6] Eidelman Y and Andreev S G Biophysical study of the globular organisation of interphase chromosomes 2002 Radiat. Prot. Dosim. 99 217–8

[7] Boyle S, Gilchrist S, Bridger J M, Mahy N L, Ellis J A and Bickmore W A The spatial organization of human chromosomes within the nuclei of normal and emerin-mutant cells 2001 Hum. Mol. Genet. 10 211–9

[8] Loucas B D and Cornforth M N Complex chromosome exchanges induced by gamma rays in human lymphocytes: an mFISH study 2001 Radiat. Res. 155 660–71

[9] Andreev S G and Edel’man Iu A Globular model of interphase chromosome and intrachromosomal exchange aberrations 1999 Radiats. Biol. Radioecol. 39 10–20

[10] Zhang Y, McCord R P, Ho Y J, Lajoie B R, Hildebrand D G, Simon A C, Becker M S, Alt F W and Dekker J Spatial organization of the mouse genome and its role in recurrent chromosomal translocations 2012 Cell 148 908–21

[11] Norbury J W et al. Galactic cosmic ray simulation at the NASA Space Radiation Laboratory 2016 Life Sci. Space Res. (Amst.) 8 38–51

[12] Oike T et al. Visualization of complex DNA double-strand breaks in a tumor treated with carbon ion radiotherapy 2016 Sci. Rep. 6 22275

[13] Scaife J E, Barnett G C, Noble D J, Jena R, Thomas S J, West C M and Burnet N G Exploiting biological and physical determinants of radiotherapy toxicity to individualize treatment 2015 Br. J. Radiol. 88 20150172

[14] Huber R, Braselmann H, Geinitz H, Jaehnert I, Baumgartner A, Thamm R, Figel M, Molls M and Zitzelsberger H Chromosomal radiosensitivity and acute radiation side effects after radiotherapy in tumour patients - a follow-up study 2011 Radiat. Oncol. 6 32