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Mechanisms of formation of ionising radiation-induced chromosomal aberrations: Impact of repair pathways and nuclear architecture

(Chromosome Structure)

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(summary)

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Introduction

Chromosomal aberrations (CA) contribute to a great extent to hereditary defects and to the development of neoplasms in humans. Ionising radiation (IR) is very efficient in inducing CA in cells both *in vitro* and *in vivo* and the analysis of CA is an important approach to assess radiation exposure. To date, molecular cytogenetic techniques have significantly increased the resolution and accuracy of detection and quantification of different types of CA. In this project we aimed at further unravelling the mechanisms of radiation-induced CA. To reach this goal we studied the events occurring from the initial DNA damage, its repair or mis-repair and the biological factors influencing the ultimate yield of CA. We employed multi-colour FISH and image analysis systems for sensitive detection of CA. Particularly, the project focussed on the impact of chromatin conformation and gene density on induction and repair of IR-induced DNA double strand breaks (DSB) and the role of these factors in the persistence of cancer-related CA. The fast increasing knowledge of the interphase nucleus gives insights in the packaging of chromosomes in territories and their spatial distribution and allows assessing the role of nuclear architecture in the formation of CA. Ultimately, this knowledge will improve our understanding of the interactions leading to CA after exposure to IR. These interactions between chromosomes are strongly influenced by DNA repair pathways and in this project we have assessed the relative contribution of the two major pathways for DSB repair, e.g. non-homologous end joining (NHEJ) and homologous recombination (HR) in the formation of CA. However, the final outcome of CA after IR is not only depending on DNA repair, but is also influenced by two other important defence mechanisms, namely cell cycle checkpoints and apoptosis. Therefore, we included in our study the analysis of the role of cell cycle checkpoints and apoptosis on the yield and types of IR-induced CA. The results obtained in this project clarify significantly the mechanisms involved in the formation of radiation-induced CA and the influence of several biological and physical factors on the ultimate yield of CA. We expect that this knowledge contributes to more rational estimates of genetic risks after exposure to IR.

Objectives

IR is very efficient in inducing CA in cells both *in vitro* and *in vivo* and DNA DSB is considered to be the most important lesion for the induction of CA. In recent years, molecular cytogenetic techniques have significantly increased the resolution and accuracy of detection and quantification of different types of CA. Along with the availability of mutant cell lines and knockout mouse models sensitive to radiation, this has offered new insights into the mechanisms of formation of CA. The present proposal is a multi-disciplinary approach aimed at further unravelling the mechanisms of radiation-induced CA by studying the events occurring from the initial DNA damage, its repair or mis-repair and the biological factors influencing the ultimate yield of CA. In particular, the project focuses on:

(a) intragenomic heterogeneity of induction and repair of DSB combining pulsed field electrophoresis and premature chromosome condensation in concert with fluorescence *in situ* hybridisation (FISH) with chromosome and region-specific DNA probes,

(b) the visualisation of radiation-induced DSB in the cell nucleus by using sensitive labelling protocols or antibodies against proteins that specifically bind to blunt or cohesive DNA ends,

(c) the relative contribution of the two major pathways of DSB repair, namely non-homologous end joining (NHEJ) and homologous recombination repair (HRR) in the formation of CA. To reach this goal, different strategies will be undertaken including the exploitation of mutant cell lines known to be deficient in specific repair pathways, investigation of different types of tissues, e.g. embryonic stem cells and splenocytes from knockout mice defective in DSB repair, and the induction of DSB exclusively in specific regions of the genome and assessment of their interactions with regions devoid of DSB,

(d) the effects of radiation on interphase nuclear architecture, e.g. specific chromosomal domains at different stages of cell cycle, the positioning of centromeres and telomeres, telomeric length and terminal fusigenic properties,

(e) the influence of cell cycle checkpoints on the yield of radiation-induced CA by employing cell lines deficient in genes controlling these checkpoints,

(f) the impact of radiation-induced apoptosis on the ultimate yield of CA,

(g) examination of radiation-induced CA concerning their application as new biomarkers for cancer risk, and

(h) the detection of CA in the whole genome by employing multi-colour FISH and image-analysis systems (such as SKY or COBRA) and estimation of radiation-induced chromosomal intrachanges, especially paracentric inversions prevalent in different types of human cancers.

The results obtained in this project are expected to clarify the mechanisms involved in the formation of radiation-induced CA and the influence of several biological and physical factors on the ultimate yield of aberrations observed.

Results

In this project we have assessed the mechanisms that are involved in the formation of CA starting from initial damage up to the final quantification of CA. Initial chromosomal damage in the interphase nucleus can be determined with the premature chromosome condensation (PCC) technique, whereas immunostaining of DNA double strand breaks (DSB) or pulse field electrophoresis provide tools to measure DSB directly. It is obvious that PCC does not detect all IR-induced DSB and might contain DNA breaks from apoptotic cells. A fraction of IR-induced DSB is repaired very fast and this might represent repair of damage possibly related to free radicals-induced DNA lesions in open chromatin. The slow-repairing fraction is unlikely to be related to complex damage (clustered damage) induced by free radicals as have been proposed from modelling studies. Immunostaining of histone H2aX-P provides a very sensitive tool to measure DSB particularly at low dose of X-rays and this method is now frequently applied to measure DSB at single-cell level. However, currently it is unclear whether this approach leads to reliable estimates of repair of DSB.

It is almost a dogma in the field that initial damage and its processing might be influenced by chromatin structure. This hypothesis was intensively tested in this project and it appeared that gene density or chromatin conformation has no significant influence on the formation of CA.

Therefore we conclude that these factors are not of importance for retrospective bio-dosimetry.

In this project two novel findings have been made regarding the formation of CA. Firstly, repair of DNA damage generated by ^{125}I decay (local multiple-damage sites) leads to CA that involved the damaged chromosome as well as undamaged chromosomes. This indicates that CA can be formed by a single DSB, e.g. by lesion/non-lesion interactions. Such a mechanism is quite controversial in view of overwhelming evidence that two hits are required to form CA. Secondly, elucidation of the 3-D distribution of chromosomes in interphase nuclei of mammalian cells revealed that the positioning of gene-rich and gene-poor chromosomes predicts the outcome of CA in X-irradiated lymphocytes. This provides for the first time evidence for the correctness of the proposed nuclear model and the biological impact of nuclear organisation on CA formation. Moreover, analysis of X-ray-induced CA also showed a non-random distribution (and interactions) between other pairs of chromosomes. Although we found that IR does not induce global changes in nuclear organisation of chromatin (not even in the damaged region), it induces local changes in chromatin involving a redistribution of heterochromatin in chromosomes 1 and 9. The current hypothesis is that this redistribution of heterochromatic regions is part of a stress response. The mechanism underlying this response is unknown but genetic factors (BRCA2 and XPF) are involved.

Accumulating evidence was generated in this project that radio-sensitivity affects the length of the telomeres (ends of chromosomes) and vice versa, e.g. that shortening of telomeres leads to radio-sensitivity. This observation was made both in *in vitro* studies as well as in *in vivo* (breast cancer patients). Different mechanisms may account for the telomeres shortening and instability. Firstly, it was shown that the presence of a single chromosomal rearrangement in the mouse genome causes significant but random telomeres shortening. Secondly, it is obvious that genes involved in IR response also play a role in maintenance of telomere length. Hence, structural chromosomal alterations (translocations) and repair defects may affect telomeres stability.

Experiments with human lymphocytes provided direct evidence that the frequencies and types of CA after radiation are modulated by cell cycle checkpoints and apoptosis. Suppression of these two defence mechanisms increased the progression of damaged cells through G2 and affected the outcome of CA. Particularly, the Msh2 protein (involved in mismatch repair) and Werner syndrome helicase play a critical role in the repair of IR-induced DSB by homologous recombination in the S phase (stalled replication) and G2 phase respectively. Clear evidence was provided that apoptosis affects the outcome of CA after IR. Moreover, apoptotic response appeared to be associated with cells carrying unstable aberrations (such as dicentrics). Using various molecular probes, the quantification and qualification of CA has reached a high level of resolution allowing to detect efficiently a variety of inter- and intrachanges. Persistent chromosomal insertions appeared to be a hallmark of exposure to high LET irradiation. The research that has been granted by this proposal has resulted in a total of 59 peer-reviewed publications.

Implications

Mechanistic understanding of radiation responses, particularly those related to the formation of CA, will improve the estimation of genetic risk after ionising radiation exposure. In recent years it has been stated that chromatin structure affects induction and processing of IR-

induced DNA damage, but in this project no evidence was found for a significant role of chromatin structure neither in processing of damage nor in the persistence of CA. The important conclusion is that these factors are not of importance for retrospective bio-dosimetry and CA measurements based on total genomic content are reliable for exposure and risk estimation.

With regard to repair of DSB, the experiments revealed evidence for two different mechanisms that might be of direct relevance to genetic effects of IR. Firstly, DSB formed by ^{125}I decay might be repaired by lesion/non-lesion interactions suggesting that this type of clustered damage is repaired by strand invasion of a non-damaged chromosome. For risk assessment this has important implications as under certain conditions (e.g. possibly depending on the complexity of the damage) a single DSB may give rise to translocations.

In this project another mechanism that appeared to be of importance for the formation of CA became manifest. Detailed analysis of the formation of CA provided direct experimental evidence that the homologues of the gene-dense chromosome 19 are proximal to each other, leading to preferential exchanges between these homologues. Also other chromosomes turned out to be preferentially engaged in exchanges. We consider this as an important direction of research for future radiobiology. The relevance of nuclear organisation with respect to cancer was recently demonstrated by an elegant study by Haigis et al. (*Nature Genetics* 33 (2003), 33) demonstrating that changes in spatial organisation of DNA sequences by translocation affects the frequency of LOH for the *Apc* tumour suppressor gene. It is obvious that this could be an alternative mechanism by which radiation could influence the process of recombination (e.g. by stimulating LOH) and can lead to cancer. In addition, IR-induced CA might contribute to telomere instability as the presence of a single chromosomal rearrangement in the mouse genome causes significant but random telomere shortening. Telomere shortening increases chromosomal fusions: a hallmark of instability that might play an important role in the process of cancer as well. Last but not least, advanced molecular cytogenetics has identified chromosomal insertions as signature of high LET radiation exposure.