Mutation Research xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

Mutation Research/Genetic Toxicology and Environmental Mutagenesis

journal homepage: www.elsevier.com/locate/gentox
Community address: www.elsevier.com/locate/mutres

Stress induced by premature chromatin condensation triggers chromosome shattering and chromothripsis at DNA sites still replicating in micronuclei or multinucleate cells when primary nuclei enter mitosis

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ARTICLE INFO

Article history:
Received 24 July 2015
Accepted 25 July 2015
Available online xxx

Keywords:
Premature chromosome condensation
Chromosome aberrations
Micronuclei
Chromosome shattering
Chromothripsis

ABSTRACT

Combination of next-generation DNA sequencing, single nucleotide polymorphism array analyses and bioinformatics has revealed the striking phenomenon of chromothripsis, described as complex genomic rearrangements acquired in a single catastrophic event affecting one or a few chromosomes. Via an unproven mechanism, it is postulated that mechanical stress causes chromosome shattering into small lengths of DNA, which are then randomly reassembled by DNA repair machinery. Chromothripsis is currently examined as an alternative mechanism of oncogenesis, in contrast to the present paradigm that considers a stepwise development of cancer. While evidence for the mechanism(s) underlying chromosome shattering during cancer development remains elusive, a number of hypotheses have been proposed to explain chromothripsis, including ionizing radiation, DNA replication stress, breakage–fusion–bridge cycles, micronuclei formation and premature chromosome compaction. In the present work, we provide experimental evidence on the mechanistic basis of chromothripsis and on how chromosomes can get locally shattered in a single catastrophic event. Considering the dynamic nature of chromatin nucleoprotein complex, capable of rapid unfolding, disassembling, assembling and refolding, we first show that chromatin condensation at repairing or replicating DNA sites induces the mechanical stress needed for chromosome shattering to ensue. Premature chromosome condensation is then used to visualize the dynamic nature of interphase chromatin and demonstrate that such mechanical stress and chromosome shattering can also occur in chromosomes within micronuclei or asynchronous multinucleate cells when primary nuclei enter mitosis. Following an aberrant mitosis, chromosomes could find themselves in the wrong place at the wrong time so that they may undergo massive DNA breakage and rearrangement in a single catastrophic event. Specifically, our results support the hypothesis that premature chromosome condensation induces mechanical stress and triggers shattering and chromothripsis in chromosomes or chromosome arms still undergoing DNA replication or repair in micronuclei or asynchronous multinucleate cells, when primary nuclei enter mitosis.

1. Introduction

First insights into the central role of chromosomes in cancer development emerged essentially early in the twentieth century from studies by Boveri. Examining cancer cells under the microscope, he observed the presence of peculiar chromosomes and this led to the proposal that cancers are abnormal clones of cells characterized and caused by abnormalities of hereditary material [1]. At present, one consistent hallmark of human cancer genomes are numerical and structural alterations in chromosomes caused by erroneous repair of DNA double-strand breaks (DSBs) and include deletions, duplications, inversions and translocations [2–9]. Chromosome instability is indeed a central aspect of cancer cell biology and has been proposed as a driving mechanism for the generation of genomic rearrangements, which may result in complex patterns of regional copy number changes [10,11]. These alterations have the potential to interrupt or activate multiple genes and, in turn,

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http://dx.doi.org/10.1016/j.mrgentox.2015.07.014
1383-5718/

produce changes in cell function that can give rise to successive waves of clonal expansion, which ultimately yield the phenotypes of malignant cancer cells [12,13]. The key feature of this stepwise model for cancer development, which is the most widely accepted model, is the progressive accumulation of genomic changes that result in the loss of tumor suppressor functions, the activation of oncogenes and /or the generation of fusion genes with oncogenic potential [14,15].

Recently, the combination of next-generation DNA sequencing, single nucleotide polymorphism array analyses and bioinformatics revealed the striking phenomenon of chromothripsis, described as complex genomic rearrangements acquired in a single catastrophic event affecting one or a few chromosomes [16]. In this striking phenomenon, contiguous chromosomal regions are fragmented into many pieces and then randomly fused together by the cell's DNA repair machinery. In contrast to the stepwise paradigm for the development of cancer, chromothripsis is considered as a single-step catastrophic event and currently examined as an alternative mechanism of oncogenesis. However, the evidence for the mechanism(s) underlying chromosome shattering in a single event remains elusive. Among the different hypotheses proposed, two of them presuppose the formation, through errors in mitosis, of a micronucleus which contains the chromosomes that may undergo shattering [17,18]. In the first, chromosome shattering occurs by mitotic entry, with the micronucleus still undergoing DNA replication. In the second, defective DNA replication in the micronucleus initiates serial, microhomology-mediated template switching that produces local rearrangements with altered gene copy numbers. Interestingly, experimental evidence supporting the first hypothesis was provided 40 years ago, demonstrating, particularly, that premature chromosome condensation (PCC) induces shattering of chromosomes still undergoing DNA replication within micronuclei, when primary nuclei enter mitosis [19,20]. Recently, it has been shown that chromosome shattering might arise from an error in mitotic chromosome segregation that leads to the production of a micronucleus (MN). Thus, DNA rearrangements and mutations acquired in MN could be incorporated into the genome of a developing cancer cell [17]. At present, however, the exact mechanisms responsible for chromosome shattering and chromothripsis remain controversial [15]. It is postulated that chromosome shattering is produced in a single catastrophic event via an unknown mechanical or cellular stress during DNA replication within the micronucleus [15,18].

In the present work, we attempt to provide experimental evidence for chromosome shattering and its biological basis, in order to better understand how localized chromosome shattering may take place in a single catastrophic event, which is required for chromothripsis to ensue. Considering the dynamic nature of the nucleoprotein complex in chromosomes, capable of rapid unfolding, disassembling and refolding, we examine the chromosome condensation process, through histone phosphorylation, as the origin of a mechanical stress that causes chromosome shattering localized at the DNA damage repairing or replicating sites. Premature chromosome condensation (PCC) is used to visualize the dynamic nature of interphase chromatin [21] and to show that such mechanical stress and chromosome shattering can also occur in chromosomes trapped in micronuclei or asynchronous multinucleate cells. Specifically, we hypothesize that, following an aberrant mitosis, chromosomes or chromosome arms still undergoing DNA replication could find themselves in the wrong place at the wrong time within micronuclei or asynchronous multinucleate cells. As a result, these chromosomes will undergo PCC and chromosome shattering, causing massive DNA breakage and rearrangement in a single catastrophic event, when primary nuclei enter mitosis. We raise, therefore, the question of whether the occurrence of such a unique cellular effect may underlie the hallmark of chromothripsis, involving local chromosome shattering in a single-step catastrophic event.

2. Materials and methods

2.1. Cell cultures

Peripheral blood from healthy individuals was drawn in heparinized tubes. Informed consent was obtained from each donor. Cultures were set up by adding 0.5 ml of whole blood to 5 ml of RPMI-1640 medium (Gibco), supplemented with 10% fetal bovine serum (FBS), 1% Phytohaemagglutinin (PHA), 1% glutamine and antibiotics (penicillin: 10,000 U/ml; streptomycin: 10,000 μg/ml (Biochrom)). Chinese hamster Ovary (CHO-K1) cells, (kindly provided by Prof George Iliakis, University of Duisburg-Essen, Germany), were grown at their 20th passage in McCoy's 5A (Biochrom) culture medium, supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine and antibiotics, incubated at 37 °C in a humidified atmosphere with 5% CO_2. CHO cultures were maintained as exponentially growing monolayer cultures in 75 cm^2 plastic flasks, at an initial density of 4×10^5 cells/flask. Colcemid (Gibco) at a final concentration of 0.1 μg/ml was added to CHO cultures for 4 h and the accumulated mitotic cells were harvested by selective detachment. Once a sufficient number of mitotic cells had been obtained, they were used as suppliers of the mitosis promoting factors (MPF) needed for cell fusion and PCC induction in human lymphocytes or CHO cells. To restrain CHO cells from proceeding to S-phase, depleted conditioned medium (C-medium), which had been collected from 10 days old CHO cultures, was used. CHO mitotic cells were allowed to divide in this depleted conditioned medium and cultured for 12 h at 37 °C, before trypsinizing and fusing them with mitotic CHO cells to induce PCCs and analyze them in G_1-phase.

2.2. Irradiation conditions and autoradiography

Irradiation was carried out in a Gamma Cell 220 irradiator (Atomic Energy of Canada Ltd., Ottawa, Canada) at room temperature and at a dose rate of 40 cGy/min. In experiments involving autoradiography, cells were pre-incubated for 0-30 min with ^{3}H-thymidine (^{3}H-TdR) (2 μCi/ml, specific activity 72.5Ci/mmol). Prepared slides were allowed to dry overnight at room temperature and were subsequently immersed in photographic emulsion. Slides were dried in a slight draft and placed in light tight boxes at 4 °C for 2–5 days. After this period of time the slides were developed and analyzed. In experiments involving G_2-checkpoint abrogation following 1 Gy of γ-irradiation, a final concentration of 4 mM caffeine was used as we have described earlier [22,23].

2.3. Premature chromosome condensation

To isolate human lymphocytes, whole blood was layered on top of an equal amount of Ficoll-Paque gradient in a test tube, before centrifugation for 20 min at about 1800 rpm. The isolated lymphocytes were kept in culture medium RPMI-1640, supplemented with 10% FBS, 1% glutamine and antibiotics. Cell fusion and induction of PCCs using polyethylene glycol (PEG) was performed essentially as described previously [24,25]. Briefly, for each fusion, 1/3 of the mitotic cells that had been harvested by shake-off from a 75 cm^2 flask of exponentially growing CHO cells, were mixed with lymphocytes obtained from 0.5 to 1 ml blood in serum-free RPMI-1640 medium in a 15 ml round-bottom culture tube, in the presence of colcemid. After centrifugation at 1000 rpm for 6 min, the supernatant was discarded without disturbing the cell pellet, keeping the tubes always inverted in a test tube rack on a paper towel

Please cite this article in press as: G.I. Terzoudi, et al., Stress induced by premature chromatin condensation triggers chromosome shattering and chromothripsis at DNA sites still replicating in micronuclei or multinucleate cells when primary nuclei enter mitosis, Mutat. Res.: Genet. Toxicol. Environ. Mutagen. (2015), http://dx.doi.org/10.1016/j.mrgentox.2015.07.014