Ionizing radiation (IR)-induced DNA damage and repair from 3D-genomic perspective

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Introduction
IR (see Glossary), a typical exogenous DNA damage inducer and a commonly occurring carcinogen, causes double-strand breaks (DSBs), the most lethal form of genome damage (Figure 1A). Its effects on biological genetic material occur within the 3D chromatin structure. However, the existing omics techniques are insufficient to fully probe these effects.

With the development of sequencing technologies and microscopy-based techniques, the complex 3D organization of genomes is being recognized. The 3D structure of chromatin and the mechanisms underlying the regulation of gene expression, cell proliferation and differentiation, body development, and disease occurrence and development are gradually being discovered [1]. This information will promote further exploration of higher chromatin structures. An increasing amount of research in radiobiology and related fields is being performed to examine the relationship between IR and the 3D genome.

For example, the spatial and genomic distribution of IR-induced DSBs was found to better simulate DNA damage processes [2].

Known IR-related carcinogenic mechanisms consist of abnormal overexpression of some proto-oncogenes and downregulation of tumor suppressor genes. Coding genes account for less than 3% of the genome, and most variations occur in noncoding regions. Using the 3D genome approach to examine long-range interactions between enhancers and promoters, IR-induced mutation targets in the whole genome can be considered, enabling the exploration of new carcinogenic mechanisms. Whether IR induces variation in the 3D chromatin structure, which in turn leads to abnormal regulation of gene expression, and ultimately to cell carcinogenesis and tumorigenesis, warrants exploration.

IR effects on 3D genomes with different hierarchical structures
The genetic functional elements of living organisms, including coding genes, noncoding genes, and cis-regulatory elements, form complex 3D spatial structures. DNA is wound around histones to form nucleosomes, which form nuclear fibers. Further, loops and topologically associated domains (TADs) boundaries are enriched in CCCTC-binding factor (CTCF) and cohesin binding sites (Figure 1).

Different hierarchical chromatin structures respond differently to IR. Experiments have shown that cells with fewer nucleosomes (the fundamental units of chromatin) accumulate more DNA damage due to IR and conversely, that nucleosomes can protect DNA from IR damage [3]. In the absence of such protection, genes in open chromatin regions (OCRs), representing transcriptionally active euchromatin, are more susceptible to IR damage (Figure 1B) [3].

Glossary
A and B compartments: the A compartment is associated with open chromatin on behalf of active transcriptional euchromatin. The B compartment is associated with closed chromatin on behalf of the inactive transcriptional heterochromatin.
CCCTC-binding factor (CTCF): this prominent transcription factor contributes to the regulation of transcription, recombination, chromatin architecture, and TADs.
Double-strand breaks (DSBs): these breaks are the most significant form of damage to genetic material, leading to cell death if not repaired and to chromosomal translocation, an etiology of carcinogenesis, if misrepaired.
Etoposide: this anticancer chemotherapy agent, a typical inducer of exogenous DSBs, inhibits TOP2, thereby stopping DNA replication and inducing cell cycle arrest, apoptosis, and autophagy.
Homologous recombination (HR): HR is a form of DNA repair in which the genetic material is exchanged between homologous DNA strands. During this process, homologous DNA sequences are used as templates to repair damaged DNA sequences.
Ionizing radiation (IR): this radiation consists of particles, X-rays, or gamma rays with sufficient energy to cause ionization of the medium through which it passes.
Lamina-associated domains (LADs): these domains are typically repressive regions in the genome. At the nuclear peripheries, chromatin associations with the nuclear lamina aid in functional genome organization.
Liquid–liquid phase separation (LLPS): this process involves the self-organization of macromolecules, including proteins and nucleic acids, into distinct membraneless and liquid-like compartments via multivalent interactions involving hydrophobic and electrostatic contacts. The detection of various multivalent interactions in LLPS bodies has enabled the distinction of these bodies from other molecular complexes or clusters.
Non-homologous end joining (NHEJ): a DNA repair pathway that directly joins broken DNA ends to non-homologous template strands.
Nucleus-associated domains (NADs): these domains are generally characterized by low gene density and expression. Heterochromatin in eukaryotic interphase cells frequently localizes to the nuclear peripheries.
Open chromatin regions (OCRs): these special regions of the human genome are accessible through DNA regulatory elements and are characterized by high levels of transcriptional and replication activity.
Topoisomerase II (TOP2): TOP2 is involved in chromatin organization, nucleic acid metabolic processes, and regulation of DNA metabolic processes. It is located in terminal DNA replication regions and synaptonemal complexes.
Topologically associated domains (TADs): these genomic regions are defined by a high degree of self-interaction between spatially proximal DNA.
Chromatin loops mediate the interaction of regulatory elements such as enhancers with promoters to regulate gene expression in a precise temporal and spatial manner. Loop anchors at domain boundaries bind to CTCF in the direction of major convergence [1,4]. This phenomenon has been demonstrated experimentally that hotspots of DNA damage and translocation have been observed near chromatin loop anchors [5]. Further, endogenous DSBs are produced during DNA replication and transcription and are correlated with greater fragility at loop anchors that have greater fragility due to the presence of topoisomerase II (TOP2) [5,6]. The probability of DSBs occurrence due to TOP2 association increases with transcriptional activity [5]. Etoposide, a well-known inducer of exogenous DSBs, coincides with TOP2B binding and activity sites [5], which colocalize with CTCF [6]. Although the major exogenous DSBs peak is known to be adjacent to loop anchors, no research work has examined the relationship between loop anchors and IR-induced fragility up till now. So, there is reason to believe that there may be some degree of correlation between loop anchors and IR-induced fragile sites (Figure 1C) (see the supplementary information online).

TADs are highly self-interacting regions in the genome and local coregulated genes frequently appear in the same TAD [1]. The current model of TAD formation suggests that loop extrusion of the cohesin complex dynamically aggregates the genomic region in TADs, until it is blocked by CTCF [4]. Decreased interaction across TAD boundaries, following IR exposure, indicates increased segregation of TADs. TAD boundary strengthening has been observed not only in aggregates but also at specific loci (Figure 1D) [7].

Although UV light and other external carcinogens induce DNA damage, somatic mutation frequencies are similar in LADs and NADs [9,10]. However, whether chromatin near the nuclear membrane or NADs is more susceptible to IR remains controversial and needs to be investigated further using radiobiological and 3D genome approaches.

Thus, the available evidence shows that OCRs and TAD boundaries are more susceptible to IR damage and are strongly associated with loop anchor location. Based on existing research conclusions, it can be hypothesized that A compartments are more susceptible to IR and that B compartments, LADs, and NADs may have radiation resistance.

**Figure 1.** Effects of ionizing radiation (IR) on 3D genome structure at different hierarchies. (A) IR causes double-strand break (DSB) in DNA. (B) Open chromatin regions (OCRs) are more vulnerable to IR than the nucleosome clusters area. (C) At the loop level, loop anchors may be vulnerable to IR. (D) Topologically associated domain (TAD) boundary is vulnerable to IR. (E) At the genomic compartment level, A compartment may be more vulnerable to IR than B compartment. (F) Lamina-associated domains (LADs) and nucleolus-associated domains (NADs) may have invulnerability to IR. Abbreviation: CTCF, CCCTC-binding factor.
Effects of the 3D genome structure on repair following IR-induced damage

Following IR damage, cell initiates the repair mechanism. Recently, Stanic and Mekhail [9] have summarized the integration of DNA damage responses with dynamic spatial genome organization. The 3D genomic structure and liquid–liquid phase separation (LLPS) of DNA repair factors cooperate to promote the mobility and repair of damaged DNA. Furthermore, as nucleosome density affects chromatin mobility, the nucleosome structure acts as a barrier to genome repair [11]. A high degree of heterochromatin compaction (as in LADs and NADs) impedes DNA damage repair by restricting access to repair machinery [8,9,12]. Thus, although these areas are less likely to be damaged, any damage that occurs in them is more difficult to repair. In addition, the nuclear layer protein B1, in LADs, is critical for DNA damage repair, resulting in a difference in repair efficiency between LADs and other areas [10].

Although most DSBs repaired by non-homologous end joining (NHEJ) tend to be anchored by the nucleoskeleton [13], some homologous recombination (HR) repair sites exist and move to the nuclear peripheries. LLPS may be a fundamental behavior of biopolymers, which are involved in a variety of biological activities, including DNA repair [8,14]. Further, 53BP1 is an important participant in regulating IR-induced DSB repair process [14], and, via LLPS, may facilitate the movement of damaged DSB terminals to the nucleolus peripheries or even into the nucleoplasm by binding to its enriched functional DNA repair factors [14]. Nuclear pores, which are abundant in the nuclear membrane region, also act as transport channels for DNA damage repair factors [10]. These factors may affect the repair processes and efficiency of LADs and NADs following IR damage.

3D genome research may inform the interpretation of radiosensitivity differences

The difference in radiosensitivity is an objective phenomenon caused by the difference in damage by IR or the difference in repair following damage. Radiotherapy is a major cancer treatment; however, its effectiveness is compromised by the unavoidable limitation that cancer cells tend to be more radioresistant than normal cells [15]. The practical significance of studying differences in radiosensitivity mechanisms lies in the application of the findings to efforts such as protection from radiation, development of antiradiation drugs, identification of radiotherapy targets, and development of new clinical diagnoses and treatment techniques.

Impairments in DSBs repair and signaling pathways may be responsible for the differences in radiosensitivity [15]. However, few studies have examined radiosensitivity from a 3D-genomic perspective. Based on the current knowledge described in the previous two sections, it can be speculated that differences in the hierarchical 3D chromatin structure are important factors leading to differences in radiosensitivity. These warrants detailed and exhaustive research on this subject.

Concluding remarks

IR damage and repair are complex processes that involve dynamic spatiotemporal changes in the whole genome. With the rapid progress in sequencing technology and 3D genomics research, related theoretical innovations are expected to occur. From the perspective of 3D genomics, IR damage and repair are different at various hierarchies of the spatial chromatin structure. Although several findings in this field are controversial (Box 1), the 3D-genomic perspective can lead to the generation of novel ideas for the study of IR damage mechanisms as well as provide theoretical support for the development of innovative clinical diagnoses, treatment techniques, and more effective countermeasures against the radiotherapy resistance of tumors.

Declaration of interests

The authors declare that they have no competing interest.

Supplemental information

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