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DNA double-strand breaks repair kinetics in mammalian and human cells after proton and nitrogen irradiation

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DNA double-strand breaks (DSBs), defined as the simultaneous damage of the two DNA strands, are considered one of the most complicated types of DNA lesions to repair due to several closely related complications – DNA DSB repair systems restrictions and non-cycling cells. There are two basic cellular mechanisms responsible for DNA DSBs recovery: non-homologues end joining (NHEJ) and homologues recombination (HR). NHEJ is considered to be the main mechanism for DNA DSBs recovery since it does not depend on the cell cycle and repairs the majority of DBS, though it is also error-prone and thus mutagenic. HR, on the other hand, tends to be more accurate; nevertheless, it relies on the second copy of the DNA molecular as a template for the DNA resynthesis step, confining it to G2/M phases of the cell cycle. Providing that radiation exposure generates a lot of DNA DSBs, it is clear that most of them repaired via NHEJ might be repaired incorrectly leading to mutations. However, the DNA damage response is well-tuned and has not precisely been determined in different cell and tissues. For instance, neuron stem cells (NSCs) are incredibly sensitive to genetic mutations making the accurate DNA DSBs repair essential as far as other cell lineages originate in them. It has been shown that stem cells contain 10-times less genetic mutations in comparison with other cycling somatic cells. Whereas non-cycling mature brain neurons are able to withstand the substantial number of DNA damages without disastrous outcomes for brain functions. Moreover, another example of cycling tumor cells might enter the cell cycle with unrepaired DNA DSBs that leads to mutations; yet it does not prevent tumors'growth. All three examples revealing the complexity of DNA repair machinery in distinctive cell types.

In our work, we compare several cell lines of mammalian and human cells derived from different types of tissues to explore the DNA DSBs repair kinetics after exposure to protons or N15-ions: the cell lines of normal human dermal fibroblasts (NHDF), mouse glioblastoma cells (U87), human melanoma cells (B16), and rat primary hippocampal cell culture (mature hippocampal neurons, astrocytes, NSCs). To analyze the DNA DSBs repair kinetics, the immunofluorescent staining method was utilized with gH2AX or 53BP1 as protein markers of DNA DSBs. Mature neurons, astrocytes and NSCs in the primary hippocampal cell culture were identified by using the cell-type specific markers as MAP2, GFAP, and nestin, correspondingly.

It is shown that the maximum formation of radiation-induced gH2AX and 53BP1 foci in NHDF, B16 and U87 cell lines is revealed at 1 h after proton or nitrogen-irradiation with 1.25 Gy dose. Whilst the peak number gH2AX or 53BP1 foci in the hippocampal cell culture cells shifts towards 4 h post-irradiation. This might demonstrate the difference in DNA repair machinery involved into the DSBs recovery in different cells and tissues, especially in NSCs after nitrogen-irradiation. It was pointed out that nestin-positive cells revealed the lowest number of 53BP1 foci among all tested cell lines. Supposing that NSCs should possess meticulous DNA DSB repair systems, these results might be indicative of the bigger role of HR pathway since 53BP1 protein is known for its role in NHEJ restricting the access to DNA DSB ends from HR proteins.

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