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Review

Spatiotemporal characterization of ionizing radiation induced DNA damage foci and their relation to chromatin organization

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ABSTRACT

DNA damage sensing proteins have been shown to localize to the sites of DNA double strand breaks (DSB) within seconds to minutes following ionizing radiation (IR) exposure, resulting in the formation of microscopically visible nuclear domains referred to as radiation-induced foci (RIF). This review characterizes the spatiotemporal properties of RIF at physiological doses, minutes to hours following exposure to ionizing radiation, and it proposes a model describing RIF formation and resolution as a function of radiation quality and chromatin territories. Discussion is limited to RIF formed by three interrelated proteins ATM (Ataxia telangiectasia mutated), 53BP1 (p53 binding protein 1) and γH2AX (phosphorylated variant histone H2AX), with an emphasis on the later. This review discusses the importance of not equating RIF with DSB in all situations and shows how dose and time dependence of RIF frequency is inconsistent with a one to one equivalence. Instead, we propose that RIF mark regions of the chromatin that would serve as scaffolds rigid enough to keep broken DNA from diffusing away, but open enough to allow the repair machinery to access the damage site. We review data indicating clear kinetic and physical differences between RIF emerging from dense and uncondensed regions of the nucleus. We suggest that persistent RIF observed days following exposure to ionizing radiation are nuclear marks of permanent rearrangement of the chromatin architecture. Such chromatin alterations may not always lead to growth arrest as cells have been shown to replicate these in progeny. Thus, heritable persistent RIF spanning over tens of Mbp may reflect persistent changes in the transcriptome of a large progeny of cells. Such model opens the door to a "non-DNA-centric view" of radiation-induced phenotypes.

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Abbreviations: RIF, radiation-induced foci; DSB, double strand break; IR, ionizing radiation; Post-IR, following exposure to ionizing radiation; ATM, Ataxia telangiectasia mutated; ATMp, ATM phosphorylated at serine 1981; γ-H2AX, histone H2AX phosphorylated at serine 139; 53BP1, p53 binding protein 1; PFGE, pulse field gel electrophoresis; LET, linear energy transfer (typical unit: keV/μm); HZE, ions with high energy and high atomic number.

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1. Introduction

A well accepted paradigm in radiation biology is that ionizing radiation (IR) induced DNA double strand breaks (DSB) are the most deleterious form of DNA damage. It is thought that unrepaired DSB lead to death and misrepaired DSB may lead to viable chromosomal rearrangements. Some of these rearrangements may be instrumental in the development of cancer. DSB happen regularly in cells as consequences of cell exposure to external insults or internal metabolism, such as, oxidative stress or DNA replication errors. Thus cells have evolved efficient and rapid repair responses to maintain the integrity of the genome. Sensor proteins are thought to detect the presence of a DSB, and then recruit transducer proteins which provide the signals to enzymes to repair the break. Depending on the severity of the damage and the cell cycle status of the damaged cell, sensor proteins, also modified by transducers, will induce either cell cycle delay for repair, programmed cell death or senescence.

Sensor proteins have been shown to localize to the sites of DSB within seconds to minutes following IR exposure, resulting in the formation of microscopically visible nuclear domains referred to as radiation-induced foci (RIF). In mammalian cells, Rad51 protein was

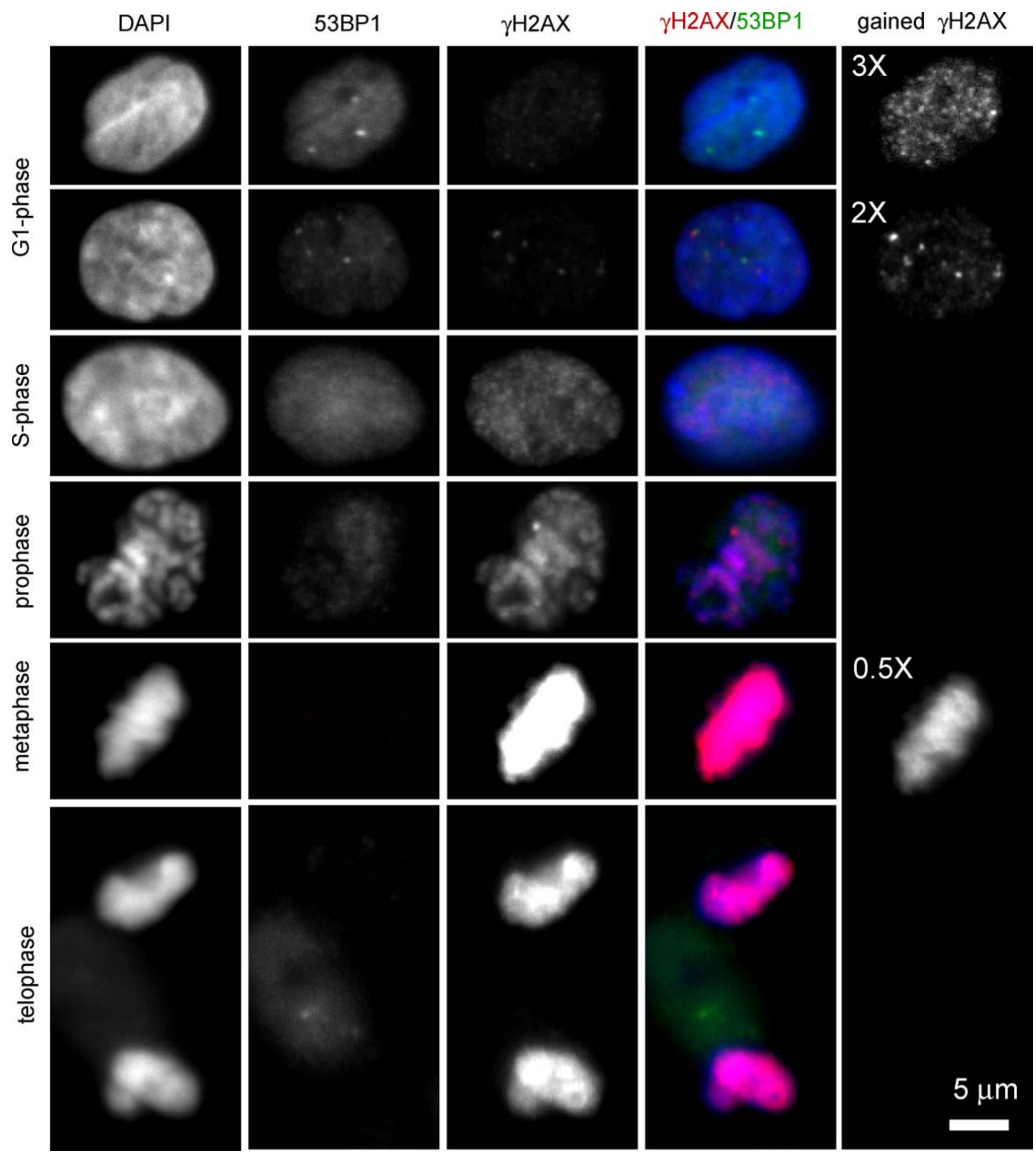

Fig. 1. Example of typical γH2AX/53BP1 dual staining in cycling normal non-irradiated human mammary epithelial cells (MCF10A). In these images, as previously described [26], γH2AX has been fluorescently labeled in red with mouse monoclonal anti phospho-histone H2AX (Ser139) antibody (1.42 μg/ml; lot #27505; Upstate Cell Signaling Solutions Inc. Charlottesville, VA) and secondary Alexa 594 (at 1:300 from Molecular Probes, Invitrogen, Carlsbad, CA). 53BP1 has been fluorescently labeled in green with rabbit polyclonal anti 53BP1 (5 μg/ml, lot #A300-272A, Bethyl Lab, Montgomery, TX) and secondary Alexa 488 (at 1:300 from Molecular Probes, Invitrogen, Carlsbad, CA). Cells have been counter stained with DAPI which labels nuclear DNA (blue). Each channel represents one center slice of a cell acquired with the same exposure time and digital camera gain. Each row depicts a different phase of MCF10A, going from G1 (top) to mitosis (bottom). G1 cells typically show no γH2AX foci or few bright γH2AX foci. However, if the γH2AX channel gain is increased by a factor 3, the presence of many dim foci is then visible (upper right panel). In contrast, 53BP1 shows a pattern in G1 that typically matches DAPI signal, with some spontaneous foci as well. DAPI and 53BP1 pattern similarity disappears during S-phase, even though 53BP1 signal remains uniform and elevated. γH2AX immunoreactivity is significantly increased during S-phase with a pattern similar to the dim foci revealed by gained enhancement in G1. As cells move to mitosis, γH2AX immunoreactivity further increases as depicted with a fully saturated signal in metaphase that needs to be acquired with half the gain in order to not saturate the image. γH2AX pattern in mitosis matches DAPI, revealing full phosphorylation of H2AX in the condensed chromosomes. In contrast, 53BP1 seems to be progressively excluded from the nucleus during mitosis.

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