Persistent DNA damage foci, cellular senescence and low dose radiation

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Ionizing radiation (IR) induced DNA double-strand breaks (DSBs) are cytologically detectable as large nuclear foci that contain phosphorylated histone H2AX (γH2AX), the adaptor protein 53BP1, and several other proteins that participate in the sensing and processing of DNA damage (DNA damage foci). In normal human cells, moderately high IR (0.5-1 Gy) doses cause the rapid appearance of these foci (acute DNA damage foci), which gradually disappear over 24 h, prior to the resumption of cell proliferation. Higher IR doses (5-10 Gy) cause a similar rapid appearance and disappearance of DNA damage foci, but >99% of the cells retain 3 or more of these foci which persist for many weeks after irradiation. Cells irradiated at these doses fail to resume proliferation and enter into a permanent p53 dependent non-dividing state termed cellular senescence. Moreover, the presence of these persistent foci correlates strongly with the senescent secretory phenotype, which is thought to adversely affect the surrounding tissue. We find that priming human cells with low doses of IR (0.5-2 cGy) prior to a challenge with a high IR dose accelerates the rapid disappearance of acute DNA damage foci, suppresses the appearance of persistent DNA damage foci, and suppresses the induction of cellular senescence. To understand the mechanisms by which low priming doses of IR suppress senescence, we carried out a series of *in situ* biochemical assays to determine the nature of the persistent DNA damage foci. Upon treatment of fixed cells with a mixture of phosphatases, both acute and persistent damage foci lost γH2AX immunostaining, but not the co-localizing 53BP1 immunostaining. Purified DNA-dependent protein kinase (DNA-PK) reconstituted γH2AX immunostaining in acute DNA damage foci (1-2 h after moderate or high doses of IR), consistent with the existence of DNA DSBs in these foci. However, DNA-PK failed to reconstitute γH2AX immunostaining in persistent DNA damage foci (>24 h after high doses of IR), suggesting that these persistent foci are structurally distinct from the acute foci at early time points. Moreover, replication protein A (RPA) transiently associated with persistent, but not acute, DNA damage foci, and activated p53 (phosphorylated on serine 15) stably associated with the persistent, but not acute, foci. Our findings raise the possibility that persistent DNA damage foci are sites of locally altered chromatin which maintain p53 signaling to maintain the senescence phenotype. Low IR doses either, prevent, retard the formation of, or facilitate the resolution of these persistent foci, which in turn may facilitate abrogation of the senescence response.