

FOCI OF DNA DAMAGE

Olga A. Sedelnikova, Izumi Horikawa, Drazen B. Zimonjic, Nicholas C. Popescu, William M. Bonner (P), and J. Carl Barrett

Laboratory of Molecular Pharmacology, Laboratory of Biosystems and Cancer, Laboratory of Experimental Carcinogenesis, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, 20892

The DNA double-strand break (DSB) is the Dr. Jekyll/Mr. Hyde of genome integrity. On the one hand DSB formation is essential in several normal cellular processes, but on the other, accidental DSB formation may lead to permanent genomic damage, to cancer, and to death. A universal cellular response to a DSB is the phosphorylation of several thousand molecules of a histone protein H2AX to form γ -H2AX in the chromatin flanking the break site.

Immunocytochemical analysis with anti- γ -H2AX reveals the number and position of each nuclear DSB as a focus of γ -H2AX (γ - focus). Unstressed cells are not devoid of γ -foci. Most cells of tumor lines generally exhibit >3 foci per nucleus, but some tumor lines exhibit manyfold more. Unstressed normal human cells exhibit increasing numbers of γ -foci as they senesce---from ~0.4 per early passage cell to >2 per late passage cell---and similar increases in the incidence of γ -foci occur in both the somatic and germline tissues of mice as they age (Sedelnikova et al., *Nature Cell Biology*, 6:168, 2004). In both mice and human cells, these foci are sites of accumulation for DNA DSB repair proteins, indicating that they are sites of DSBs and repair. These γ -foci of unknown origin are termed cryptogenic foci. When nascent DSBs are generated in human cell cultures with ionizing radiation, the numbers of nascent γ -foci are similar in cells at different stages of senescence but the rates of dimensional focal growth and focal accumulation of DSB-repair proteins are substantially slower in late passage cells and even slower in cells taken from Werner Syndrome patients, who exhibit premature aging and genome instability. These findings support the notion that the ability of cells to repair DNA damage may decrease with age. Radiation-induced DSBs are repaired in young and old cultures, but age-related cryptogenic DSBs are not, indicating that these lesions may be complex and unrepairable. Young fibroblast cultures induced to prematurely senesce by exposure to DNA damaging agents exhibit increases in DSB incidence similar to those found in replicative senescence, indicating that accumulating unrepairable DSB-containing lesions may be a casual factor in aging. The results demonstrate that mammalian cells aging in vitro and in vivo accumulate unrepairable DSB-containing lesions that may play a causal role in aging.