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Irreversible cellular senescence induced by prolonged exposure to H_2O_2 involves DNA-damage-and-repair genes and telomere shortening

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Abstract

H_2O_2 has been the most commonly used inducer for stress-induced premature senescence (SIPS), which shares features of replicative senescence. However, there is still uncertainty whether SIPS and replicative senescence differ or utilize different pathways. 'Young' human diploid fibroblasts (HDFs), treated with prolonged low doses of hydrogen peroxide, led to irreversible cellular senescence. Cells exhibited senescent-morphological features, irreversible G1 cell cycle arrest and irreversible senescence-associated β-galactosidase positivity. The appearance of these cellular senescence markers was accompanied by significant increases of p21, gadd45 expression and p53 binding activity, as well as a significant decline in DNA repair capability and accelerated telomere shortening. Our results suggest that multiple pathways might be involved in oxidative SIPS, including genes related to DNA-damage-and-repair and telomere shortening, and that SIPS shares the same mechanisms with replicative senescence in vivo. Our findings indicate that several aging theories can be merged together by a common mechanism of oxidative damage, and that the level of oxidative DNA-damage-and-repair capacity may be exploited as reliable markers of cell senescence.

Keywords: Cellular senescence; Oxidative stress; Hydrogen peroxide; DNA damage; DNA repair; Telomere; Human fibroblast

Abbreviations: SIPS, stress-induced premature senescence; HDF, human diploid fibroblast; PD, population doublings; SA-β-gal, senescence-associated β-galactosidase; SCGE, single cell gel electrophoresis; MV-MDD, mean value of migration distance of DNA; UDS, unscheduled DNA synthesis; EMSA, electrophoretic mobility shift assay; TRF, terminal restriction fragment

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1. Introduction

Human diploid fibroblasts (HDFs) exhibit finite proliferative potential in vitro, the so-called Hayflick limit (Hayflick & Moorhead, 1961). They undergo a limited number of population doublings (PD) before entering a state of permanent growth arrest, referred to as "replicative senescence," "cellular senescence" or "cellular aging" (Campisi, 1996; Campisi, Kim, Lim, & Rubio, 2001), in which they remain alive and metabolically active but are completely refractory to mitogenic stimuli. HDFs offer the typical model for studying the process of aging in vitro. Various oxidative stresses have been used to study the onset of cellular senescence, such as exposure of cells to ultraviolet (UV) light (Rodemann, Bayreuther, Francz, Dittmann, & Albiez, 1989), hyperoxia (Honda, Hjelmeland, & Handa, 2001), *tert*-butylhydroperoxide (*t*-BHP) (Toussaint, Houbion, & Remacle, 1992), and H_2O_2 (Frippiat et al., 2001; Frippiat, Dewelle, Remacle, & Toussaint, 2002). The early onset of cellular senescence induced by these stresses is termed stress-induced premature senescence (SIPS) (Toussaint, Medrano, & von Zglinicki, 2000). H_2O_2 has been the most commonly used inducer of SIPS, which shares features of replicative senescence: similar morphology, senescence-associated β-galactosidase activity, cell cycle regulation, etc. (Chen & Ames, 1994; Dimri et al., 1995; Frippiat et al., 2001, 2002). However, what triggers SIPS and whether it shares the same mechanisms and pathways with replicative senescence are still not well understood.

DNA damage is by far the most detrimental consequence of oxidative stress. It causes a series of irreversible dysfunctions and eventually cell death (Kawanishi, Hiraku, & Oikawa, 2001). Through a common p53-dependent mechanism, DNA damaged cells may undergo death, usually by apoptosis, or growth arrest (Itahana, Dimri, & Campisi, 2001); but alternatively, cells can also repair damaged DNA by complex enzymatic mechanisms like base excision repair, nucleotide excision repair, mismatch repair, etc. (de Boer & Hoeijmakers, 2000). Cellular senescence may occur if DNA damage is not serious enough to induce cell death but DNA damage cannot be completely repaired (Beckman & Ames, 1998). In fact, it was found that the levels of oxidative DNA damage are significantly increased in senescent cells, especially in post-mitotic tissues (Hamilton et al., 2001), and that the levels are correlated with aging and age-related diseases (Bayens, 2002; Mattson, 2003). Meanwhile, the DNA repair capacity related to oxidative DNA damage significantly declined in senescent cells (Parrinello et al., 2003). Therefore, the DNA repair capacity and the accumulation of DNA damage play important roles in the initiation and the process of replicative senescence.

However, controversy exists as to whether the DNA damage observed in senescent cells reflects prolonged oxidative attack (Hall et al., 2001), a decline in repair mechanisms (Klungland et al., 1999; von Zglinicki, Burkle, & Kirkwood, 2001), or a combination of these factors. Also, arguments exist regarding whether oxidative SIPS shares the same mechanisms and pathways with replicative senescence. Some research has shown that senescent phenotypes induced by sub-lethal doses of H_2O_2 are uncoupled from telomere shortening (Chen, Prowse, Tu, Purdom, & Linskens, 2001) which is another theory of aging. To elucidate the underlying mechanism of oxidative SIPS, i.e., what triggers it, how similar it is to replicative senescence, and whether the oxidative damage and DNA repair capacity could be considered biomarkers of aging, we designed experiments to induce SIPS by H_2O_2 treatment and observed the pathways involved, and compared these with replicative senescence. We treated 'young' human diploid fibroblasts (2BS) with prolonged low doses of hydrogen peroxide instead of using acute treatment by sub-lethal doses of H_2O_2 in order to mimic chronic oxidative stress under pathophysiological conditions. We then evaluated oxidative DNA damage and DNA repair capacity after the oxidative damage in 2BS cells using the single cell gel electrophoresis (SCGE) assay and the unscheduled DNA synthesis (UDS) assay, and then correlated the levels of these two markers to cellular senescence monitored by proliferation rate and senescence-associated-β-galactosidase (SA-β-galactosidase) expression. We also examined the role of DNA damage genes and telomere shortening to explore the relationship between DNA-damage-and-repair and telomere shortening during oxidative damage induced SIPS.

2. Materials and methods

2.1. Cell culture and treatment protocols

Human diploid fibroblasts 2BS, derived from embryonic human lung primary culture (obtained from