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Stress-induced premature senescence and tissue ageing

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Abstract

Various human proliferative cell types exposed *in vitro* to many types of subcytotoxic stresses undergo stress-induced premature senescence (SIPS). The known mechanisms of appearance the main features of SIPS are reviewed: senescent-like morphology, growth arrest, senescence-related changes in gene expression. All cell types undergoing SIPS *in vivo*, are likely to participate in the tissular changes observed along ageing. For instance, human diploid fibroblasts exposed *in vivo* and *in vitro* to pro-inflammatory cytokines display biomarkers of senescence and might participate in the degradation of the extracellular matrix observed in ageing.

Keywords: Ageing; Stress; Senescence

1. Introduction

Many proliferative cell types like lung and skin human diploid fibroblasts (HDFs), human melanocytes, endothelial cells, human retinal pigment epithelial cells, exposed to subcytotoxic stress (UV, *tert*-butylhydroperoxide (*t*-BHP), H_2O_2, ethanol, mitomycin C, hyperoxia, γ-irradiations, homocysteine, hydroxyurea, etc.) undergo stress-induced premature senescence (SIPS) *in vitro*. SIPS can be defined as the sustained effects of subcytotoxic stress on proliferative cell types, including irreversible growth arrest of (a majority of) the cell population (for a review see [1]). The first studies were based on the hard work performed by Bayreuther's group who defined seven morphological types (''morphotypes'') that successively appear during *in vivo* and *in vitro* ageing of human diploid fibroblasts. It was shown there was a sharp shift from the youngest morphotypes to the oldest ones after a variety of subcytotoxic oxidative stresses (for a review see [2]).

2. Mechanisms of SIPS-associated morphological changes

Several leads exist to explain the SIPS-associated morphological changes. First the presence of the retinoblastoma protein (Rb) is necessary for the appearance of the senescent-like morphology after subcytotoxic H_2O_2 stress. HDFs expressing the papilloma virus E7 protein (which facilitates the proteolytic degradation namely of Rb) and exposed to H_2O_2 do not adopt a senescent-like morphology. In HDFs expressing mutated E7 proteins unable to bind Rb, a senescent-like morphology is observed after H_2O_2 stress. The appearance of stress fibres in H_2O_2-treated HDFs occurs together with a redistribution of vincullin and paxallin [3,4]. Transforming growth factor-β1 (TGF-β1) is overexpressed after subcytotoxic H_2O_2 stress. Incubations of H_2O_2-treated HDFs with antibodies against TGF-β1, or against TGF-β receptor II abrogate the stress-induced appearance of the senescent-like morphology. The overexpression of TGF-β1 disappears in HDFs expressing E7 [5].

3. Other biomarkers of senescence in SIPS

The percentage of HDFs positive for senescence associated β-galactosidase activity (SA β-gal) increases after repeated subcytotoxic stress with *t*-BHP. The commonest age-related deletion of mitochondrial DNA is also detected in these conditions. Several genes undergoing senescence-related changes in expression level are also differentially expressed in SIPS [6,7]. For instance, apolipoprotein J, fibronectin and osteonectin are overexpressed in both replicative senescence and SIPS [6] while c-*fos* is down-

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regulated in both situations [7,8]. The retrovirus-mediated stable overexpression of apo J increases the survival of WI-38 HDFs after exposure to cytotoxic concentrations of *t*-BHP and ethanol. In addition, it decreases the induction of the senescence-like morphology and SA β-gal activity after exposure to subcytotoxic ethanol or *t*-BHP concentrations [9].

Stimulation of IMR-90 HDFs with TGF-β1 triggers the appearance of biomarkers of SIPS such as SA β-gal activity and increased mRNA steady-state level of the senescence-associated genes fibronectin, osteonectin, and apolipoprotein J. Antibodies against TGF-β1 or TGF-β1 receptor II abrogate the overexpression of these genes observed after subcytotoxic H_2O_2 stress, and the stress-induced appearance of SA β-gal activity. The expression of fibronectin, osteonectin, and apolipoprotein J disappears in HDFs overexpressing E7 [5].

TGF-β1 induces the release of H_2O_2 from IMR-90 HDFs within 8 hr following exposure. Diphenyliodonium, an inhibitor of NADPH oxidase complex and other flavoproteins, inhibits this TGF-β1-induced H_2O_2 production [10]. Thereby a constant oxidative stress might be generated once TGF-β1 is overexpressed, which could explain why cells in SIPS are kept in a state of irreversible growth arrest.

No mitogenic response is observed in H_2O_2-induced SIPS after incubation with serum or growth factors [11]. H_2O_2-treated HDFs are blocked mainly in the G1 phase of the cell cycle, and cells in G2 are also observed [3]. Hyperoxia under 40% O_2 also leads to a growth arrest of HDFs mostly in the G1 phase [12]. Hypophosphorylation of Rb was found that was triggered by an overexpression of the cyclin-dependent kinase inhibitor (CDKI) $p21^{waf-1}$ [3,6].

When IMR-90 HDFs are treated for 2 hr with 50–200 μM H_2O_2, a dose-dependent fraction of HDFs detach at 16–32 hr after the treatment. The cells remaining attached are growth arrested and develop SIPS. The detached cells show dose-dependent caspase-3 activation and typical morphological changes associated with apoptosis. Apoptotic cells are mainly distributed in the S-phase of the cell cycle, while growth-arrested cells exhibit G1- and G2/M-phase distributions. H_2O_2 pretreatment induces G1 arrest and prohibits induction of apoptosis by a subsequent H_2O_2 challenge. Reduction of p53 level with human papillomavirus E6 protein prohibits the activation of caspase-3 and decreases the proportion of apoptotic cells. Growth arrested cells have elevated $p21^{waf-1}$, while $p21^{waf-1}$ is absent in apoptotic cells [13].

4. Does SIPS exist *in vivo*?

It seems that cells in SIPS might affect *in vivo* tissue (patho)physiology during the course of ageing. HDFs excised from gastric venous ulcers display several features of senescent cells: reduced proliferative capacity, enlarged size, SA β-gal activity, and overexpression of fibronectin. TNF-α is a major component identified in the fluid of these ulcers. Exposure of HDFs to TNF-α leads to appearance of a senescent-like phenotype [14–16]. Several reports show that pro-inflammatory cytokines induce the degradation of the extracellular matrix [17–19]. These data are puzzling since the overexpression of several metalloproteinases is also observed in senescent HDFs (for a review see [20]). Moreover, human ageing is accompanied by an elevation of the circulating levels of TNF-α and IL-1 (for a review see [21]). SA β-gal activity positive cells are also found in arteries subjected to balloon angioplasty, chronic hepatitis, tissue surrounding liver carcinomas and benign prostatic hyperplasia (for a review see [22]).

5. Conclusion

The appearance of SIPS could be due to exacerbated modifications of a limited number of parameters that also undergo, to a more limited extent, age-related changes, among multiple other age-related changes. Irreversible changes in gene expression take place when SIPS becomes established: some genes become permanently underexpressed while others become overexpressed. A change in the cellular targets under positive feed-back is operated by the establishment of cascades of new regulatory loops, eventually locking the system in a new attractor [23]. Common and different pathways are induced after exposure to different kinds of subcytotoxic stress, changing the level of expression of common and different genes. Some of these pathways seem to share common portions with replicative senescence. Abnormal oxidative stress is involved in many inflammatory processes, pathologies and intoxications. It would be worth examining whether cells taken from inflammatory sites are more prone to SIPS, thereby favouring the ‘‘inflamm-ageing’’ theory of ageing [24].

Lastly complex interactions might exist between senescent cells and surrounding normal or cancer cells, whether fibroblasts or not. On one hand, the senescence of fibroblasts suppresses their own tumorigenesis. On the other hand, senescent fibroblasts, whether in replicative senescence, oncogene overexpression-dependent senescence or H_2O_2-induced SIPS, were shown to promote the growth and tumorigenesis of neoplastic and proneoplastic epithelial cells, and not of normal epithelial cells [25].

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