EDITORIAL



Increasing the complexity of respiratory syncytial virus infection: Reactive oxygen species, DNA damage, and premature senescence

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Introduction

Human respiratory syncytial virus (hRSV) is the major cause of respiratory tract infection in infants and young children, and it is estimated that close to 100% of children have faced a hRSV infection when reaching the age of 2. In 0.5 to 2% of the cases, hospitalization is required. Complications sometimes severe and life threatening can occur, particularly in groups at risk such as infants born prematurely or with a pre-existing health condition. Thus, hRSV is an important health problem worldwide with an economic impact estimated higher than influenza.¹⁻⁵

In the present issue of Virulence, Martinez *et al.* describe for the first time that hRSV is able to trigger the premature senescence of its target cells. Formation of DNA damage, including DNA double-strand breaks was observed and linked to the entry of cells into senescence. Moreover, a marked increase in reactive oxygen species (ROS) production is recorded in infected cells and constitutes the trigger for DNA damage formation and senescence induction.⁶

Replicative senescence and stress-induced premature senescence

The so-called "Hayflick limit" indicates that normal somatic cells can achieve a finite number of divisions, e.g. typically 50 for human fibroblasts, before undergoing an irreversible growth arrest. Replicative senescence is not synonym of cell death. Indeed, senescent cells remain alive and metabolically active for a long period of time.⁷⁻¹¹ Senescent cells display different characteristics such as an enlarged and flattened morphology ("egg on a plate"), expression of the senescence-associated β -galactosidase

activity (SA- β gal activity), accumulation of lipofuscin, presence of DNA damage, multiple changes in gene expression, presence of heterochromatin foci and secretion of pro-inflammatory cytokines and other factors.⁷⁻¹¹ Replicative senescence is also referred to as telomere-initiated senescence. Due to the end-replication problem, telomeres undergo a shortening at each cell cycle S phase, establishing a counting mechanism or mitotic clock.⁹⁻¹⁴ Telomeres end in a specialized circular structure stabilized by the shelterin complex. While, in long telomeres, the extremity of the chromosome is fully masked inside this structure, a critically short telomere becomes dysfuntional and generates a robust DNA damage response.⁹⁻¹⁴

Normal cells can also reach in a matter of days an irreversible post-mitotic state when exposed to different stress. This process has been called stress-induced premature senescence (SIPS). Remarkably, replicative senescence and SIPS share many features, including morphological changes and expression of the SA- β gal activity.¹⁵⁻¹⁸ Cells enter SIPS following a variety a detrimental stimuli (Fig. 1).^{19,20} Moreover, SIPS is independent of telomere shortening. Cells that have escaped replicative senescence, such as cancer cells or normal cells immortalized by introduction of telomerase, can still undergo SIPS in response to oxidative stresses or agents that trigger DNA damage formation such as UV and anticancer drugs like topoisomerases inhibitors.²⁰⁻²²

Central to both replicative senescence and SIPS is the activation of the DNA damage response (Fig. 1).^{10,11,19,20} Development of both stress-induced and telomere-initiated senescence has been associated with activation of the transcription factor and tumor suppressor p53 and its effector $p21^{waf-1}$. Other major actors of senescence include $p16^{INK4a}$ and pRb. The proteins $p21^{waf-1}$ and

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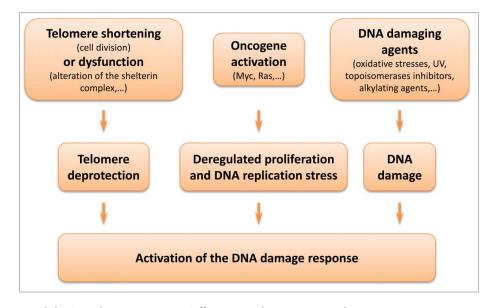


Figure 1. Senescence and the DNA damage response. Different stimuli can trigger replicative senescence or stress-induced premature senescence through activation of the DNA damage response. Telomere shortening at each DNA replication or alteration of the shelterin complex (e.g., depletion of TRF2, Telomeric repeat-binding factor 2) leads to the unmasking of chromosomes extremities. Oncogene activation leads to deregulated proliferation, promoting replication errors. Oxidative stresses and various other agents can trigger extensive non-telomeric DNA damage.

p16^{INK4A} are both cyclin-dependent kinase (CDK) inhibitors. CDK inhibition prevents pRb phosphorylation, and consequently expression of E2F target genes and cell progression into S phase.^{10,11,19,20}

hRSV-induced senescence: Friend or foe?

Martinez et al. describe in this issue that hRSV causes in vitro a marked increase of ROS in infected cells, in line with previously published studies. In the two cell lines examined, this oxidative stress produces a strong activation of the DNA damage response as noted by phosphorylation of the ATM (Ataxia Telangiectasia Mutated) kinase, induction of p53 and its effector p21^{waf-1}, as well as presence of γ H2AX nuclear foci.⁶ The antioxidants Nacetyl cysteine and reduced glutathione or overexpression of catalase exert a protective effect. The cascade of molecular events generated ultimately leads to the entry of infected cells into premature senescence (Fig. 2). Interestingly, a similar process seems to occur in vivo: DNA damage (detection of γ H2AX) is observed in the airway epithelium of mice infected with hRSV as well as expression of p16.^{INK4a6}

As discussed above, the DNA damage response is central to induction of senescence. In this respect, telomere-initiated or stress-induced senescence are tumor suppressor mechanisms that prevent the proliferation of "damaged" cells and constitute a barrier against cancer development. Critically short telomeres are prone to association and favor genomic abnormalities. Stresses that induce SIPS are highly detrimental to cell integrity (oncogene activation, exposure to a DNA damaging agent, oxidative stress) (Fig. 1). Therefore, induction of senescence by hRSV can be seen as a host defense mechanism, instructing "damaged" cells to cease proliferation.^{10,11,19,20} However, excessive accumulation of senescent cells in a tissue can also have a negative impact on its homeostasis. Senescent cells secrete a number of factors able to modulate cell growth, create a pro-inflammatory micro-environment or remodel the extracellular matrix.⁹

The airway epithelium contains stem cell populations able to proliferate in response to damage, ensuring repair and thus tissue homeostasis. In the lung ciliated pseudostratified columnar epithelium, basal cells are believed to be pluripotent stem cells able to differentiate in both ciliated cells and non-ciliated secretory cells. In addition, following damage to the ciliated cells, non-ciliated secretory cells can self-renew and differentiate into ciliated cells. In the lung alveolar epithelium, composed of type 1 and type 2 cells, type 2 cells are thought to be the progenitor of type 1 cells.²³⁻²⁷ Moreover, although it was previously thought that ciliated cells were the target of hRSV, recent works indicate that basal stem cells can also be infected.²⁸ Can we accurately demonstrate in vitro and in vivo that hRSV can infect and trigger the senescence of specific airway epithelium stem cell populations? Second, could hRSV infection and subsequent stress-induced senescence lead to a reduced capacity of these

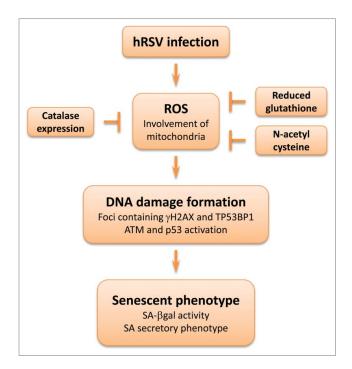


Figure 2. hRSV-induced premature senescence. Cell infection by hRSV leads to generation of ROS, including superoxide anions derived from mitochondria. The oxidative stress is sufficient to trigger DNA damage as noted by the detection of nuclear foci containing TP53BP1 (tumor protein p53 binding protein 1) and γ H2AX (in presence of DNA damage, histone variant H2AX is phosphorylated on serine 139 by the ATM kinase or other kinases). Ultimately, cells develop the senescent phenotype. Antioxidants can protect against this process: N-acetyl cysteine, catalase expression or reduced glutathione decrease the oxidative stress as well as DNA damage formation.

populations to self-renew, differentiate and maintain tissue homeostasis? Indeed, somatic stem cells also age and there are examples of decline of their function in different organs. For instance, aged skeletal muscles contain about 50% less satellite cells than healthy adult muscles, indicating a depletion of this somatic stem cell population during organismal aging.²⁹

Further questions

The article of Martinez et al. adds a new layer of complexity to the pathophysiology of hRSV infection.⁶ Causes and consequences of hRSV-induced senescence could be further investigated. For instance, how hRSV causes an increase of ROS from a mitochondrial origin? Does senescence favors or rather opposes propagation of the infection? And could hRSV-induced senescence be linked to complications sometimes observed in patients?

Furthermore, will the damage alter on the long term the normal functioning of the airway epithelium, or render it more vulnerable to future aggressions? In the article of Martinez *et al.*, it is interesting to note that DNA damage and p16^{INK4a} expression persist *in vivo* long after the acute phase of the infection, when epithelial cells are negative again for hRSV antigens.⁶ This suggests that the infection leaves a scar that may have a long-term impact on the function of the tissue. Senescent cells accumulate with age in our tissues, and although this is still intensely debated, there is a growing amount of evidence that it contributes to organismal aging as well as aging-associated pathologies.

A better understanding of the pathophysiology of hRSV infection, including a thorough knowledge of the molecular mechanisms involved, is important for a better management of the disease and hopefully the design of new therapeutic strategies to combat it.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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