Molecular Cell
Article



# Necessary and Sufficient Role for a Mitosis Skip in Senescence Induction

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http://dx.doi.org/10.1016/j.molcel.2014.05.003

## SUMMARY

**Senescence is a state of permanent growth arrest and is a pivotal part of the antitumorigenic barrier in vivo. Although the tumor suppressor activities of p53 and pRb family proteins are essential for the induction of senescence, molecular mechanisms by which these proteins induce senescence are still not clear. Using time-lapse live-cell imaging, we demonstrate here that normal human diploid fibroblasts (HDFs) exposed to various senescence-inducing stimuli undergo a mitosis skip before entry into permanent cell-cycle arrest. This mitosis skip is mediated by both p53-dependent premature activation of APC/C$^{Cdh1}$ and pRb family protein-dependent transcriptional suppression of mitotic regulators. Importantly, mitotic skipping is necessary and sufficient for senescence induction. p16 is only required for maintenance of senescence. Analysis of human nevi also suggested the role of mitosis skip in in vivo senescence. Our findings provide decisive evidence for the molecular basis underlying the induction and maintenance of cellular senescence.**

## INTRODUCTION

The inability of cultured human cells to proliferate indefinitely, ending in cellular senescence, was first described by Hayflick and Moorhead (1961). Subsequently, several lines of evidence revealed that cellular senescence was also triggered by diverse genotoxic stimuli, including telomere dysfunction, activated oncogenes, reactive oxygen species (ROS), and DNA damage (Kuilman et al., 2010). Senescence is now believed to play a critical role in suppression of tumorigenesis as well as in aging-related changes in various organs resulting from a permanent loss of proliferation capacity (Campisi and d'Adda di Fagagna, 2007; Halazonetis et al., 2008).

Cellular senescence requires functional p53 and pRB family proteins, both of which regulate growth signaling. This may explain why these genes are often mutated in a vast majority of human cancers (Burkhart and Sage, 2008; Levine and Oren, 2009). This is supported by the fact that viral oncoproteins that can inhibit either p53 or pRB family proteins allow cells to bypass cellular senescence (Shay et al., 1991). Although the precise roles of these tumor suppressors in the senescence process are incompletely understood, various models of senescence induction have been proposed (Adams, 2009; Courtois-Cox et al., 2008; Rufini et al., 2013). One such proposal is that senescence-inducing stimuli ultimately trigger a DNA damage response (DDR) that in turn activates p53. p21 (*CDKN1A*), a p53-target gene, is expressed and arrests cells at the G1 phase of the cell cycle by preventing phosphorylation and inactivation of pRB through inhibition of G1 and S phase Cdk activities (Cobrinik, 2005). pRb phosphorylation is also suppressed by another Cdk inhibitor, p16 (*CDKN2A*), that is upregulated during the senescence process (Rayess et al., 2012). Hypophosphorylated pRb suppresses transcription of canonical E2F (E2F1– E2F3) target genes to arrest the cell cycle at G1 (Rowland and Bernards, 2006).

In contrast, the accumulation of G2 phase cells during replicative senescence has also been reported, arguing against the senescence model described above (Mao et al., 2012; Ye et al., 2013). In addition, p21-mediated inhibition of Cdk1 and Cdk2 was proposed to prematurely activate APC/C$^{Cdh1}$ to destroy various APC/C substrates, resulting in long-term growth arrest at G2 in response to genotoxic stress (Baus et al., 2003; Wiebusch and Hagemeier, 2010). Thus, the fundamental basis for senescence induction and the phases at which senescent cells exit the cell cycle remain controversial. The factors that determine whether cells will undergo senescence (terminal growth arrest) versus transient cell-cycle arrest, also remain largely elusive.

In this study, we have analyzed the senescence process induced by various stimuli using time-lapse live-cell imaging. We found that the majority of cells underwent a mitosis skip before permanently exiting the cell cycle. This mitotic skipping appears to be necessary and sufficient for the induction of senescence both in vitro and in vivo.

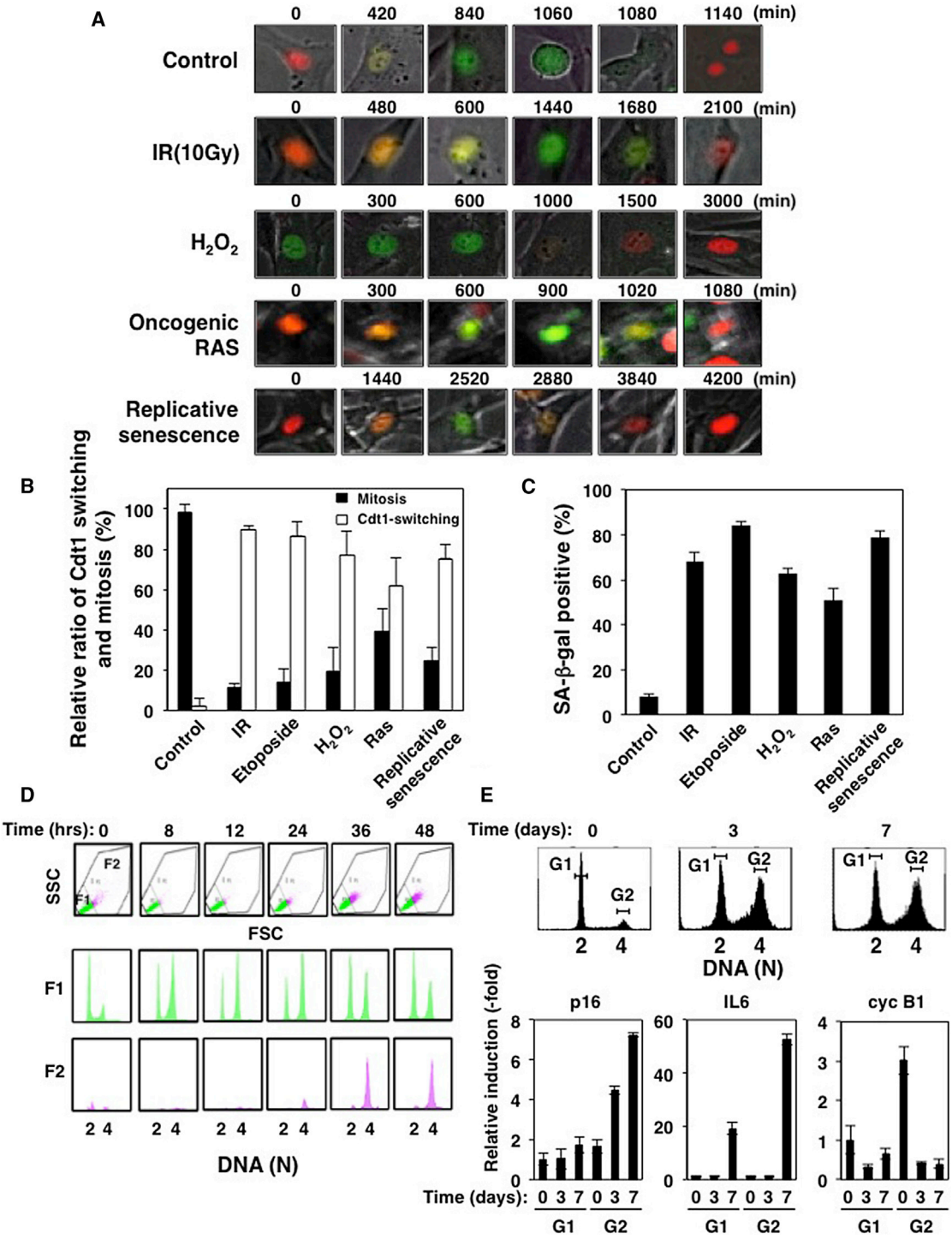


**Figure 1. Senescent Cells Are Mononucleated Tetraploid G1 Cells Triggered by a Mitosis Skip**

(A) Young (PD 8) and near senescent (PD > 60) HCA2 cells (FUCCI-HCA2 cells) were infected with FUCCI lentiviruses expressing mKO2-hCdt1 (red) and mAG-hGeminin (green). Young cells were untreated (Control) or treated with IR (10 Gy), $H_2O_2$ (50 μM) for 48 hr or etoposide (200 nM) for 48 hr. Expression of oncogenic RAS was induced by the addition of doxycycline to young cells expressing Tet-on 3×Flag-H-Ras$^{val12}$. Replicative senescence was analyzed using near senescent cells. The resulting cells were imaged 3 days after treatment. Representative images at the indicated times are shown.

(B) The relative ratio of Cdt1-switching cells versus total cells changings from green to red color was determined by counting at least 100 cells treated as in (A). Data are presented as means ±SD of at least three independent experiments.

(C) SA-β-gal-positive cells were identified using cells 6 days after treatment as in (A). Data are presented as means ±SD of at least three independent experiments.

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