Roles of Interferons and Secretory PLA₂ in Cell Senescence

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Contents

- Characteristics of cellular senescence
- Role of interferons in endothelial cell senescence
- Role of phospholipase A2 in cell senescence
Many roads to senescence in mammalian cells

Cellular senescence: an irreversible arrest of cell proliferation caused by various stresses

Replicative senescence

- Restricted proliferation of normal cells: **Hayflick limit** (Hayflick and Moorehead, 1961)
  - Organismal aging
  - Cancer suppression

- Characteristics of senescent cells
  - Enlarged and flattened morphology
  - Resistant to mitogen-induced proliferation
  - Senescence-associated $\beta$-galactosidase (SA-$\beta$-gal)
  - Altered gene expression: cell cycle regulation, **immune and inflammation**, cytoskeleton, stress response, metabolism
Senescence biomarkers

- Morphological changes
  - Larger, flat, granularity

- Biochemical changes
  - SA-β-gal activity
  - Cessation of DNA synthesis
  - p53, p16, γH2AX

- Chromatin changes
  - Senescence-associated heterochromatin foci (SAHF)
  - H3K9 trimethylation
Three Hayflick factors

Senescent phenotype: reversible?

Campisi J. Cell 120:513, 2005

A

Dysfunctional Telomeres

Oncogenes (ROS signals)

DNA Damage

\(\uparrow p53\)

Target gene transcription (e.g., \(\uparrow p21\))

Senescence growth arrest (reversible)

B

Stress

\(\uparrow p16\)

Chromatin reorganization (e.g., repress E2F target genes)

Senescence growth arrest (irreversible)
# Senescence and inflammation

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Inflammation</th>
<th>Aging</th>
<th>Calorie restriction</th>
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<tbody>
<tr>
<td><strong>Redox state</strong></td>
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<tr>
<td>ROS/RNS</td>
<td>↑</td>
<td>↑</td>
<td>R</td>
</tr>
<tr>
<td>Catalase, Superoxide dismutase</td>
<td>↓</td>
<td>↓</td>
<td>R</td>
</tr>
<tr>
<td>GSH peroxidase, GSH/GSSG</td>
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<td>↓</td>
<td>R</td>
</tr>
<tr>
<td><strong>Proinflammatory enzymes</strong></td>
<td></td>
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<tr>
<td>Inducible NO Synthase</td>
<td>↑</td>
<td>↑</td>
<td>R</td>
</tr>
<tr>
<td>Heme oxygenase-1</td>
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<td>R</td>
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<tr>
<td>Cyclooxygenase-2</td>
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<tr>
<td>Xanthine Oxidase</td>
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<tr>
<td><strong>Proinflammatory cytokines</strong></td>
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<tr>
<td>IL-1β</td>
<td>↑</td>
<td>↑</td>
<td>R</td>
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<tr>
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<td>R</td>
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<tr>
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<td>R</td>
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<tr>
<td>ICAM-1</td>
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<td><strong>NF-κB activation</strong></td>
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<td>NF-κB DNA binding activity</td>
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<td>R</td>
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<tr>
<td>NIK/IKK activation</td>
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<td>↑</td>
<td>R</td>
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<tr>
<td>Phosphorylation of IkBα</td>
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<td>R</td>
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<td>Degradation of IkB in cytoplasm</td>
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<tr>
<td>Nuclear translocation of p65 and p50</td>
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<td>NF-κB-dependent gene expression</td>
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<td>R</td>
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<td>Active MAPKs (ERK, JNK, p38 MAPK)</td>
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<td>R</td>
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</table>

Molecular Inflammation Hypothesis of Aging
(Chung, HY)

Cytokines (IL-1β, IL-6, TNFα)

NADPH oxidase
Immune cells

NF-κB activation

COX-2, iNOS

O2⁻

Epithelial cell

Redox Imbalance

Chronic Inflammation, Tissue Response

Aging

Up-regulation of inflammatory genes in replicative senescence of HDFs

Exploration of replicative senescence-associated genes in human dermal fibroblasts by cDNA microarray technology

In Kyung Yoon\textsuperscript{a}, Hyun Kyoung Kim\textsuperscript{a}, Yu Kyoung Kim\textsuperscript{a}, In-Hwan Song\textsuperscript{b}, Wankee Kim\textsuperscript{c}, Seongyong Kim\textsuperscript{a}, Suk-Hwan Baek\textsuperscript{a}, Jung Hye Kim\textsuperscript{a}, Jae-Ryong Kim\textsuperscript{a,⁎}

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure}
\caption{Expression levels of selected genes.}
\end{figure}
Aging Cell (2007) 6, pp 535–545

Regulation of replicative senescence by insulin-like growth factor-binding protein 3 in human umbilical vein endothelial cells

Kwang Seok Kim,1,2 Min-Sun Kim,1 Young Bae Seu,2 Hae Young Chung,3 Jung Hye Kim1 and Jae-Ryong Kim1

Molecular Biology of the Cell
Vol. 18, 4543–4552, November 2007

Induction of Cellular Senescence by Insulin-like Growth Factor Binding Protein-5 through a p53-dependent Mechanism

Kwang Seok Kim,*+‡ Young Bae Seu,‡ Suk-Hwan Baek,*+ Mi Jin Kim,‡§ Keuk Jun Kim,‡§ Jung Hye Kim,* and Jae-Ryong Kim*‡
Interferons and interferon-inducible genes in senescence of human endothelial cells

<table>
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<td>IFNG</td>
<td></td>
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<tr>
<td>GAPDH</td>
<td></td>
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</table>
Interferons (IFNs)

- A family of cytokines with antiviral and antiproliferative activity
- Type I, II, and III
  - Type I
    - A major component of the innate immune system
    - IFN-α: dendritic cells, monocytes, B lymphocytes
    - IFN-β: most cells (fibroblasts)
  - Type II
    - IFN-γ: lymphocytes, immunoregulatory functions
  - Type III: IFN-λ
IFN-signaling pathway

Interferons and cellular senescence

- **IFN-γ and endothelial cell senescence?**
- IFI16 (an IFN-inducible gene): up-regulated in old human fibroblasts, and induction of cell senescence by increased levels of IFI16 in old HDFs (Oncogene. 2004; 23: 6209).
- The levels of p53 mRNA and protein are increased by IFN-α/IFN-β (Nature. 2003; 424: 516), which is an important regulator of tumor suppression as well as cellular senescence.
Effect of IFNs on endothelial cell proliferation

A

- NT
- IFN-γ 500U/ml
- IFN-γ 1000U/ml
- IFN-γ 2000U/ml
- IFN-α 1000U/ml

B

- NT
- IFN-α 1000U/ml
- IFN-γ 1000U/ml

Cell proliferation (%) vs. Days

Cell count (x10^5) vs. Days
Induction of cell senescence by IFN-γ in HUVECs

A

B

IFN-α (1000U/ml)

IFN-γ (1000U/ml)

SA-β-gal staining (%)

Time (days)

0 25 50

12 18

NT

IFN-α (1000U/ml)

IFN-γ (1000U/ml)
Induction of cell senescence by IFN-γ in HUVECs

**Cell cycle**

- NT
- IFN-α (1000 U/ml)
- IFN-γ (1000 U/ml)

**Fluorescence intensity**

- ROS

**Mean fluorescence (% of control)**

- NT
- IFN-α
- IFN-γ
Induction of cell senescence by IFN-γ in HUVECs

IFN-γ induces cellular senescence in HUVECs.
IFN-γ-induced senescence via a p53 signaling pathway

A

C  p16sh  C  p53sh

p16
p53
GAPDH

B

SA-β-gal staining (%)

0  10  20  30  40

P16sh  -  -  +  +  -  -
P53sh  -  -  -  +  +  +
IFN-γ  -  +  -  +  -  +
IFN-γ-induced senescence via a p53 signaling pathway

C

p16^-/- MEF

p53^-/- MEF

NT

IFN-γ

D

SA-β-gal staining (%)

20

10

0

p16^-/-

p53^-/-

NT

IFN-γ
Post-translational modification of p53 by IFN-γ through DNA damage signaling.

Diagram showing the pathway from DNA damage to Senescence, involving ATM/ATR and p53.

- DNA damage → ATM/ATR → p53 → Senescence

Graphs showing expression of pp53s15, pATMs1981, and GAPDH over time (days 0, 3, 6, 9) for NT, IFN-α, and IFN-γ conditions.

- Graphs indicate increased expression over time, with IFN-γ showing the highest expression compared to NT and IFN-α.
B

<table>
<thead>
<tr>
<th>siATM</th>
<th>NT</th>
<th>IFN-γ</th>
<th>NT</th>
<th>IFN-γ</th>
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<tr>
<td>ATM</td>
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</tr>
<tr>
<td>pp53s15</td>
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<tr>
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<td></td>
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<tr>
<td>GAPDH</td>
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</tbody>
</table>

C

SA-β-gal staining (%)

D
Summary (IFN-γ)

- Prolonged treatment with IFN-γ induced cellular senescence in HUVECs, as confirmed by increased SA-β-gal staining, G0/G1 cell cycle arrest, and up-regulation of p53 and p21 protein levels.

- In contrast to IFN-γ, IFN-α did not induce cellular senescence in HUVECs.

- IFN-γ-induced cellular senescence was observed only in p16-knockdown cells or p16-null MEFs, but not in p53-knockdown cells or p53-null MEFs.

- Knockdown of ATM kinase rescued IFN-γ-induced cellular senescence.

- Therefore, IFN-γ might play an important role in cellular senescence through a p53-dependent DNA damage pathway.
Phospholipase A2

Hydrolysis of the sn-2 fatty acyl bond of phospholipids

Capper and Marshall, Prog Lipid Res. 40:167, 2000
PLA2 and arachidonic acid

Capper and Marshall, Prog Lipid Res. 40:167, 2000
Phospholipase A2

❖ 5 major families
  – cytosolic PLA2 (cPLA2)
  – secretory PLA2 (sPLA2)
  – Ca\(^{2+}\)-independent PLA2s (iPLA2)
  – platelet-activating factor acetylhydrolases (PAFAH)
  – lysosomal PLA2s

❖ Roles of PLA2
  – Phospholipid digestion and metabolism
  – Host defense
  – Signal transduction
  – inflammation
Secretory PLA2 (sPLA2)

- Ca\(^{2+}\)-dependent low-molecular weight (14-18 kDa) enzymes
- Released in plasma and biological fluids of patients with various inflammatory diseases
  - rheumatoid arthritis, adult respiratory distress syndrome, inflammatory bowel disease, pancreatitis and sepsis
- Increased after treatment of cells with pro-inflammatory cytokines and in diverse pathologic conditions
  - myocardial infarction, viral hepatitis, renal infarction and wound healing
sPLA2-induced effects in inflammatory cells

PLA2 and senescence

- No direct evidence between PLA2 and cell senescence

- COX-2 is up-regulated in fibroblasts during replicative senescence (Han et al., 2004; Yoon et al., 2004; Zdanov et al., 2007).

- AA treatment induces cellular senescence by enhancing COX-2 activity, and inhibition of COX-2 activity by NS-398, or by COX-2 siRNA represses cellular senescence in fibroblasts (Han et al., 2004; Zdanov et al., 2007).

- 5-lipoxygenase (LO) activity is increased during senescence-like growth arrest in fibroblasts and overexpression of 5LO promotes cellular senescence via a p53/p21-dependent pathway mediated by ROS (Catalano et al., 2005).
Inhibition of cell proliferation by sPLA2

A: Cell proliferation (% of control) vs. sPLA2 (nM)

B: Caspase-3 activity (% of control) vs. sPLA2 (nM)

C: Fluorescence intensity vs. Cell counts

Graphs show the effects of sPLA2 on cell proliferation and caspase-3 activity, with fluorescence intensity and cell counts measured in relation to sPLA2 concentration.
Induction of cell senescence by sPLA2 in HDFs

A

<table>
<thead>
<tr>
<th>125 nM</th>
<th>250 nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Y</td>
<td>O</td>
</tr>
</tbody>
</table>

B

SA-β-gal staining (%)

0 125 250 Old

sPLA2(nM)
Induction of cell senescence by sPLA2 in HDFs

C

D

Fluorescence intensity

Cell number

10^0 10^1 10^2 10^3

NT

125nM

Old

250nM
Induction of cell senescence by sPLA2 in HDFs

**E**

![Images of cells with different concentrations of sPLA2](image)

**F**

<table>
<thead>
<tr>
<th>Concentration (nM)</th>
<th>Time (days)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>2</td>
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<tr>
<td>62.5</td>
<td>4</td>
</tr>
<tr>
<td>125</td>
<td>-</td>
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<tr>
<td>250</td>
<td>+</td>
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</table>

- **pATM**
- **p53**
- **p53(ser15)**
- **p21**
- **GAPDH**
G2 arrest by sPLA2 in HDFs

A

sPLA2 (250 nM)

Cell number

Fluorescence intensity

0 d

1 d

3 d

B

Cell population (%)

0 d

2 d

4 d

G1/G0

S

G2/M

Cell number

Fluorescence intensity
Effect of sPLA2 on cellular senescence in p16-/- and p53-/- MEFs

A

<table>
<thead>
<tr>
<th></th>
<th>NT</th>
<th>sPLA2 (250nM)</th>
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<tbody>
<tr>
<td>p53-/-</td>
<td><img src="image1.png" alt="Image" /></td>
<td><img src="image2.png" alt="Image" /></td>
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<tr>
<td>p16-/-</td>
<td><img src="image3.png" alt="Image" /></td>
<td><img src="image4.png" alt="Image" /></td>
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</table>

B

![Graph showing SA-β-gal staining percentage](image5.png)

Legend:
- p53-/-
- p16-/-
Effect of sPLA2 on cellular senescence in p16-/- and p53-/- MEFs

<table>
<thead>
<tr>
<th></th>
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<tr>
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<tr>
<td>p53-/-</td>
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<td>p16-/-</td>
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ROS generation by sPLA2 and effect of NAC on the senescence phenotypes of sPLA2-treated cells

A

<table>
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<tr>
<th>NT</th>
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<th>NAC+sPLA2</th>
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<td>Rhodamine</td>
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**B**

<table>
<thead>
<tr>
<th></th>
<th>NT</th>
<th>sPLA$_2$</th>
<th>NAC+sPLA$_2$</th>
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<tr>
<td>Image</td>
<td><img src="NT.png" alt="NT Image" /></td>
<td><img src="sPLA2.png" alt="sPLA$_2$ Image" /></td>
<td><img src="NAC+sPLA2.png" alt="NAC+sPLA$_2$ Image" /></td>
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**C**

![Bar Graph](BarGraph.png)

**D**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>NAC</th>
<th>sPLA$_2$</th>
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</table>
Summary (sPLA$_2$)

- sPLA2 treatment induces cellular senescence in human dermal fibroblasts (HDFs).
- sPLA2-induced cellular senescence is observed in p16-null MEFs, but not in p53-null MEFs.
- Treatment with sPLA2 increases ROS production, and an antioxidant, N-acetylcysteine, inhibits sPLA2-induced cellular senescence.
- Therefore, sPLA2 plays a role in cellular senescence in HDFs by promoting ROS-dependent p53 activation.
Acknowledgements

- Yeungnam University
  - Jung Hye Kim MD, PhD
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  - Hae Young Chung PhD

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Thank You!