Senescence Associated β-galactosidase Staining

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Cell senescence
the physiological program of terminal growth arrest

Can be triggered by:
- alterations of telomeres (replicative), or
- different forms of stress (oncogene induced)
Features of senescence *in vitro* ¹

- Cell growth arrested with a G1 DNA content
- Do not enter S phase in response to physiologic mitogens
- Genes needed for cell cycle progression are repressed
- Genes for cell cycle arrest are upregulated

¹ Dimri GP, Proc Natl Acad Sci U S A. 1995. 92(20);
² http://www.bdbiosciences.com
Features of senescence *in vitro*

- Cell growth arrested with a G1 DNA content
- Do not enter S phase in response to physiologic mitogens
- Genes needed for cell cycle progression are repressed
- Genes for cell cycle arrest are upregulated
- Change in morphology (enlarged, flat and irregular with vacuolated, granular cytoplasm) ¹
- **Remain metabolically active** and **resist apoptosis** ²

¹ Pare R. et. al. J Clin Pathol. 2013;66(6);
² http://joshmitteldorf.scienceblog.com/2015/03/13
Potential applications

- drugs
- stresses
- radiation
- genetic manipulations
- antiaging compounds

\[\downarrow \quad \downarrow\]

*induce* or *inhibit* the appearance of senescent cells
Why study senescence (cancer) ?

Senescence can:

- Arrest proliferation of pre-neoplastic cells
- Suppress tumor growth (i.e. by initiating an immune response)
- Affect efficacy of some chemotherapeutic drugs
- Stimulate neoplastic growth (late carcinogenesis)
- Prognostic biomarker

- Cancer type
- Stage
- Molecular profile of tumor cells...
Methods to detect senescence

1) SA-β-galactosidase detection
   • Cytochemical  • Histochemical  • Fluorescence-based
     - Cells in culture   - Tissue sections (frozen in liquid N₂)

2) Other senescence-associated markers
   • Western blotting
   • Immunohistochemistry
   • Immunofluorescence
     p16  p53  p21
     SAHF (Senescence-Associated Heterochromatic Foci)
Senescence Assay (principle)

- Described in 1995
- Based on activity of lysosomal enzyme - β-galactosidase

Non-senescent cell vs. Senescent cell

- lysosome

chromogenic substrate; galactose linked to indole (analog of lactose)

1 Dimri GP, Proc Natl Acad Sci U S A. 1995. 92(20)
Senescence Assay (principle)

Based on activity of lysosomal enzyme - β-galactosidase

Non-senescent cell

Senescent cell

- lysosome

pH 4.0

X-Gal

chromogenic substrate; galactose linked to indole (analog of lactose)

Acidic β-galactosidase activity

1 Dimri GP, Proc Natl Acad Sci U S A. 1995. 92(20)
Senescence Assay (principle)

Based on **activity of** lysosomal enzyme - $\beta$-galactosidase

Non-senescent cell

Senescent cell

X-Gal (chromogenic substrate; galactose linked to indole (analog of lactose))

pH 6.0
Senescence Assay (principle)

Based on activity of lysosomal enzyme - \( \beta \)-galactosidase

Non-senescent cell

Senescent cell

\( \text{pH 6.0} \)

\( \text{X-Gal} \)

chromogenic substrate; galactose linked to indole (analog of lactose)

Senescence-associated \( \beta \)-galactosidase activity
## Senescence Assay (principle)

**Formation of blue precipitate**

<table>
<thead>
<tr>
<th></th>
<th>pH 4</th>
<th>pH 6</th>
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<tbody>
<tr>
<td>Non-senescent cells</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Senescent cells</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
Senescence Assay (procedure)

Experiment: 8+ days

A. Preparation of cells

- Plating
- Treatment (IR, drugs)
- Incubation

B. Cell fixation and staining

C. Image acquisition and analysis
Procedure

A. Preparation of cells

1) Cells plated (adhere ON)

2) Treatment

3) Incubation 5-7 days under optimal growth conditions
Procedure

B. Cell fixation and staining

1) Growth media removed
2) Wash (PBS)
3) Fix with formaldehyde
4) Wash (PBS)
5) Add β-Galactosidase staining solution (X-Gal) pH 6.0
6) Seal with parafilm & incubate ON at 37°C (no CO₂ to maintain pH)
Procedure

C. Image acquisition and analysis

1) Check staining

2) Replace staining solution with PBS

3) Acquire images and analyze (quantify)
Procedure

C. Image acquisition and analysis

Count a total of ≥400 cells per experimental condition

SA-β-gal positive cells presented as % of the total cell number

1 Azad A. Int J Radiat Oncol Biol Phys. 2014 Feb 1;88(2);
2 Kim BC. Oncol Rep. 2014 May;31(5)
More details on image acquisition, analysis and quantification of results – in the lab

Thank you