Rapid detection of cellular senescence marker SA-β-gal by a novel fluorescent probe SPiDER-βGal

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1. Introduction
Cellular Senescence, a state of irreversible growth arrest, can be triggered in order to prevent DNA-damaged cells from growing. Senescence-associated β-galactosidase (SA-β-gal), which is overexpressed in senescent cells, has been widely used as a marker of cellular senescence. Although X-gal is a well known reagent to detect SA-β-gal, these are following disadvantages: 1) requirement of fixed cells due to the poor cell-permeability, 2) low quantitative capability because of the difficulty of the determination of visual difference between stained cells and not stained cells, 3) requirement of a long time of staining. The SPiDER-βGal system we have developed allows to detect SA-β-gal with high sensitivity and ease of use. SPiDER-βGal is a new reagent to detect β-galactosidase which possesses a high cell-permeability and a high retentivity inside cells. SA-β-gal are detected specifically not only in living cells but also fixed cells by using a reagent (Bafilomycin A1) to inhibit endogenous β-galactosidase activity. Therefore, SPiDER-βGal can be applied to quantitative analysis by flow cytometry.

Cellular Senescence
Normal cells → Cellular Senescence → Cancer
- Oxidative stress
- Repeated cell division
- DNA damage
- Cancer promoting
- Cancer inhibition

Detection Mechanism of SPiDER-βGal

SPiDER-βGal Characteristics
- Live cell imaging is possible
- High sensitivity
- Good retention in cells

2. Experiments

SPiDER-βGal staining
Neutralization of Lysosome: 60 min
Staining: 30 min
- Bafilomycin A1
- SPiDER-βGal
* Bafilomycin A1 works to neutralize Lysosomal pH

OX-gal staining
Fixation: 5 min
4% PFA/PBS
Staining: 4~16 hours
Visual observation

Oy-H2AX detection (DNA damage maker)

y-H2AX (Ex: 640 nm, Em: 650-700 nm)
-primary: anti-y-H2AX, secondary: anti-rabbit Alexa647

y-H2AX signal (red fluorescence) indicates that WI-38 cells in passage 12 are senescent compare with in passage 0.

OCell viability assay according to passage number
96 well plate
- WI-38 P0, 2, 7, 9 (3.0 x 10^4 cells)
- Cell Counting Kit-8
- 37°C, 2 h
OD reading at 450 nm

OD reading (mg/dl)

0 0.5 1.0 1.5 2.0 2.5
Passage 0 1 2 3 4 5

No particular change of WI-38 cell metabolic activities were observed by using Cell Counting Kit-8 (DOJINDO, CK04) which is a colorimetric cell viability assay system.

3. Conclusion
- SPiDER-βGal utilizes for live cell imaging as well as fixed samples
- Quantitative analysis of SA-β-gal is possible (FCM)
- SPiDER-βGal enables easy procedure and rapid staining of SA-β-gal

OSA-β-gal Detection

SPiDER-βGal signal was determined according to Senescent level of WI-39.

Passage 0: Passage 11
Cell line: WI-38
SPiDER-βGal (Ex: 488 nm, Em: 500-600 nm)

Stronger SPiDER-βGal signal was determined according to Senescent level of WI-39.

SPiDER-βGal characteristics:
- Live cell imaging is possible
- High sensitivity
- Good retention in cells

SPiDER-βGal-βGal with high sensitivity and ease of use. SPiDER-βGal is a new reagent to detect β-galactosidase which possesses a high cell-permeability and a high retentivity inside cells. SA-β-gal are detected specifically not only in living cells but also fixed cells by using a reagent (Bafilomycin A1) to inhibit endogenous β-galactosidase activity. Therefore, SPiDER-βGal can be applied to quantitative analysis by flow cytometry.

βGal can be applied to quantitative analysis by flow cytometry.