

Cellular Senescence Identified by β -Galactosidase Assay and HCS

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Introduction

Cellular senescence is defined as a state in which a cell is viable and metabolically active, but ceases to divide. The two most highly studied forms of senescence include replicative senescence through cellular aging and loss of telomeres and premature senescence induced by various stressors including oncogenes and chemotherapeutics¹. Both forms of senescence remove aging and malignant cells from the cell cycle, leading to tumor suppression. The senescent phenotype displays distinct morphological characteristics where cells become enlarged and flattened with increased granularity and a vacuole-rich cytoplasm. There is no single biochemical assay that definitively confirms cellular senescence. Since senescent cells cease to divide, 5-bromo-2' deoxyuridine (BrdU) incorporation is one test that is typically performed to identify senescent cells. In addition, senescent cells display characteristic gene expression patterns with specific changes in the protein levels of various cell cycle

regulators including p53, cyclin dependent kinase inhibitors (p21, p16) and retinoblastoma protein (Rb). The most widely used assay for detection of senescent cells is the β -galactosidase assay. An increase in the lysosomal compartment of senescent cells leads to high-expression of β -galactosidase detected as blue perinuclear staining specifically at pH 6.02. Whereas presenescent and senescent cells stain equally well at pH 4 and not at all at pH 7.5, only senescent cells visibly display β -galactosidase activity at pH 6.0. Most laboratories that use the β -galactosidase assay to demonstrate senescence will publish a representative image or images of the blue perinuclear staining in a small subset of cells as compared to no visible staining in control cells. This process is not at all quantitative and researchers are then allowed to choose the "best" images that may or may not be representative of the staining in the entire well. Until now, quantitation of Brightfield images has been a long, tedious and inaccurate process

performed by microscopic analysis and manual quantitation by the researcher.

We now can demonstrate a quantitative measure of β -galactosidase staining with Brightfield imaging on the Thermo Scientific Cellomics® ArrayScan VTI HCS Reader using doxorubicin² and discodermolide³ as positive controls for senescent β -galactosidase staining as compared to DMSO control.

Materials and Methods

Cell treatment and β -galactosidase Staining

A549 cells were plated at 3.5 x 10⁵ in 6-well dishes and treated for 7 days with 50 nM doxorubicin or 25 nM discodermolide to induce senescence or DMSO vehicle control. To avoid overconfluent cell populations resulting in false positive staining², treated cells were split at day 3 before staining at day 7. We used the β -galactosidase staining protocol as previously described² with staining up to 24-36 hrs at 37°C before quantitation on the ArrayScan VTI HCS Reader.

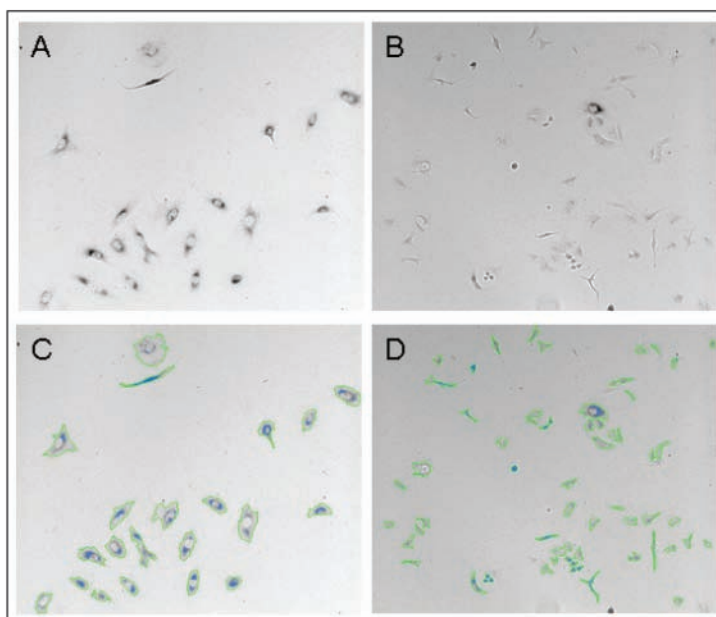


Figure 1. Automating the quantitation of β -galactosidase staining.

Images of A549 cells acquired on the ArrayScan VTI HCS Reader using the Brightfield module and analyzed with the Thermo Scientific Cellomics Compartmental Analysis BioApplication to obtain a quantitative measure of β -galactosidase staining.

(A) Brightfield image of A549 cells treated with 50 nM doxorubicin, (B) Brightfield image of A549 cells treated with 25 nM discodermolide (C and D) Algorithmic overlays applied to the images (in Figure 1A and B) quantifying the β -galactosidase staining.

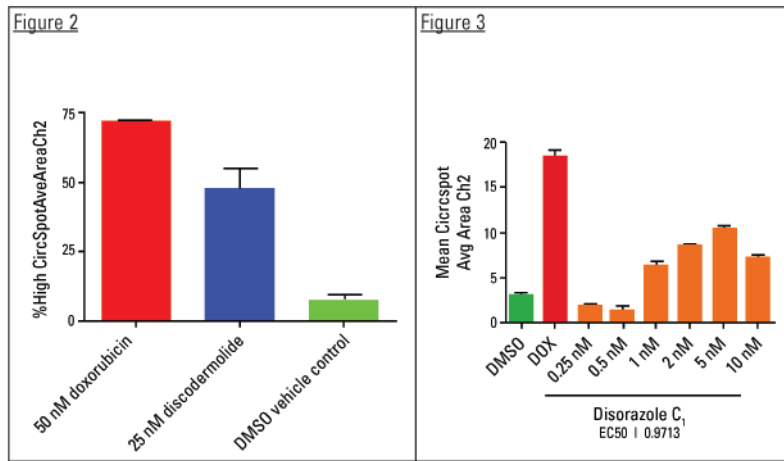


Figure 2. Automating the quantitation of β - galactosidase staining.

The graph illustrates the treatment of A549 cells with doxorubicin or discodermolide and the subsequent percent of senescent β - galactosidase staining compared to the DMSO control. Each bar represents the mean of twenty fields per well and 2 wells per condition +/- SEM.

Figure 3. Dose response of disorazole C1 compared to control conditions.

The graph shows a concentration dependent dose response of senescent β - galactosidase staining compared to the DMSO control and 50 nM doxorubicin.

Results

Figure 1 shows images of A549 cells acquired on the ArrayScan VTI HCS Reader using the Brightfield Module and analyzed with Thermo Scientific Cellomics Compartmental Analysis BioApplication to obtain a quantitative measure of β - galactosidase staining. The phenotypic results of β - galactosidase expression using Brightfield imaging are the dark spots formed in the perinuclear region of the cell. The 50 nM doxorubicin treatment (Panel A and C) show marked increase in the spot area as calculated by the bioapplication (Figure 2) over vehicle control (image not shown), whereas the 25 nM discodermolide treatment (Panel C and D) revealed less β - galactosidase expression, but still was significant over vehicle control. Figure 3 shows a quantitative dose response of disorazole treated cells compared to DMSO control and 50 nM doxorubicin.

Using the Compartmental Analysis V3 Bioapplication, Brightfield images were quantitated to determine the area of β - galactosidase staining that was present in each cell among the different treatments. DMSO vehicle control yielded few cells (7.8% of the cells) responding as β - galactosidase positive, however, both 50 nM doxorubicin (71% of the cells), and 25 nM discodermolide (48% of the cells) showed a significant population of cells that scored positive as compared to the control. A dose response curve of disorazole C1 yielded a dose dependent senescent β - galactosidase staining response and an EC50 of 0.97.

Conclusions

- A High Content Screening approach with Brightfield imaging was used to quantitate the amount of β - galactosidase expression from known positive controls of cell senescence.
- An automated approach that permits sensitive detection and quantitation of β - galactosidase expression using the ArrayScan VTI HCS Reader and Brightfield images can be employed to help increase throughput or multiplex assays with current fluorescent methods.
- Quantitation of Brightfield images for β - galactosidase expression eliminates the need for subjective, tedious and inaccurate manual microscopic analysis.

References

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