A NOVEL MULTI-MODAL PLATFORM FOR THE ACCURATE IDENTIFICATION AND ISOLATION OF SENESCENT CELLS

Annarita Scaramozza[1], Nicholas Elder[1], Xu Zhang[2], Nirvan Rouzbeh[1], Liz Murray[1], Shan Sabri[1], Shreya Deshmukh[1], Ria Gupta[1], Sina Moeinzadeh[1], Filiz Gorpe Yasar[1], Yunmin Li[1], Teresa Ai[1], Pier Federico Gherardini[1], Tarun Khurana[1], Mostafa Ronaghi[1], Nathan K. LeBrasseur[3], Gary P. Schroth[1]

[1]Cellanome, Inc. 500 Lincoln Center Drive, Foster City, CA 94404, USA
[2]Dept. of Biochemistry and Mol. Biology, Robert and Arlene Kogod Center on Aging, Mayo Clinic, Rochester, MN 55905, USA
[3]Robert and Arlene Kogod Center on Aging, Dept. of Physical Medicine and Rehabilitation, Mayo Clinic, Rochester, MN 55905, USA

INTRODUCTION

Cellular senescence is a hallmark of aging associated with age-related diseases. However, deciphering its specific role in disease is challenging, given high heterogeneity. Identification of senescent cells relies on the combination of different markers because no single marker is specific and expressed over a cell's lifecycle. Bulk approaches or methods bypassing this problem with orthogonal validation across multi-modal measurements (e.g., proteins, genes, morphology) are sub-optimal, in part, because no single technology longitudinally collects these metrics from the same, single cell. Populations identified as senescent are likely impure, making it challenging to employ fundamental cell biology approaches (e.g., cell quantification, co-cultures). Hence, there is an urgent need to decipher senescent cells at single cell resolution to enable the convergence of these multi-modal measurements.
To address these challenges, we developed a method for identifying and quantifying senescent cells by leveraging a novel cell biology platform. This platform compartmentalizes tens of thousands of single cells within permeable hydrogel enclosures, integrating imaging and nucleic acid extraction to enable linked, multi-modal analysis of the same cell across functional, phenotypic and genomic data. We validated this method to detect multi-modal hallmarks of senescence from the same, single-cells (morphology, SASP, SA-β-gal, transcriptome) and leveraged it to study primary human preadipocytes. Preadipocytes were selected as our first case study, due to their vulnerability to senescence and the significant role of adipose tissue in metabolic age-related diseases. Etoposide-treated senescent preadipocytes displayed high levels of heterogeneity across multi-modal measurements encompassing morphology, SA-β-gal, and gene expression. Multi-modal data analysis uncovered populations expressing different combinations of senescence attributes, otherwise obscured in bulk or single-modality approaches. Because this method can unambiguously link converged markers to the transcriptome, we were further able to identify shared and distinct gene signatures across these populations. Additionally, our method isolates RNA without enzymatic dissociation, avoiding selective loss of fragile senescent cells before input into current scRNA-seq technologies. These results demonstrate the potential of using this technology to generate multi-modal data across cell types and tissues to establish comprehensive benchmarks for discovering novel, specific senescence markers. We believe this method can play a meaningful role in developing consensus on the identity and utility of senescent cells which could accelerate Atlassing initiatives, efforts to discover specific markers across tissues, and / or clarify the specificity of senolytic drugs.

CURRENT CHALLENGES FOR SENESCENCE IDENTIFICATION

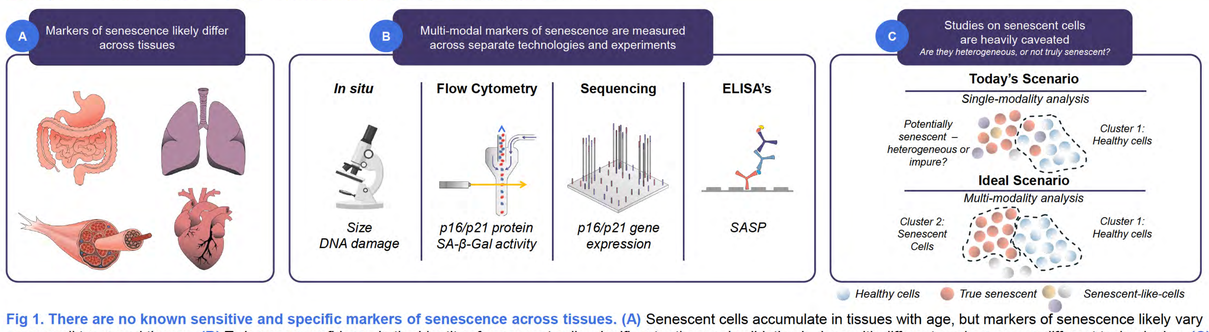


Fig 1. There are no known sensitive and specific markers of senescence across tissues. (A) Senescent cells accumulate in tissues with age, but markers of senescence likely vary across cell types and tissues. **(B)** To increase confidence in the identity of senescent cells, significant orthogonal validation is done with different markers across different technologies. **(C)** Because no single technology can collect multi-modal markers from the same, individual cells, researchers leverage single-modal markers to quantify cells, resulting in likely impure or incomplete quantification. This project aims to tackle this challenge by developing a method that measures multi-modal markers of senescence from the same, single cells.

CELLANOME TECHNOLOGY

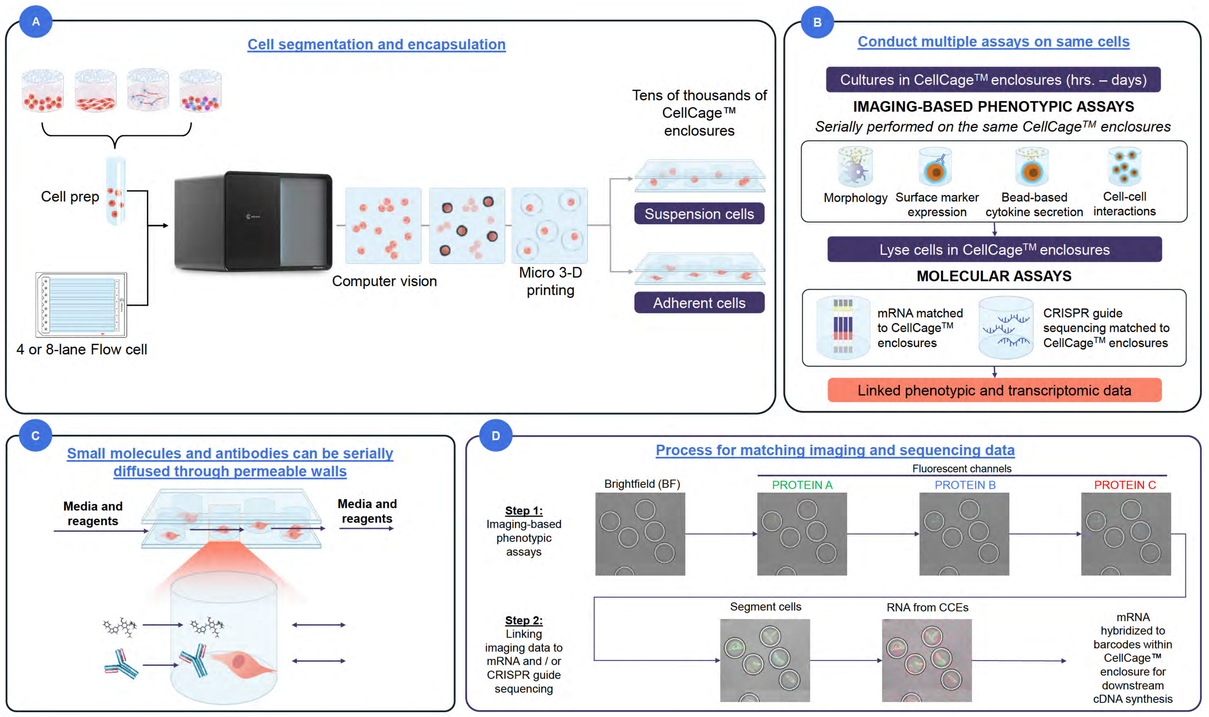


Fig 2. Cellanome's Technology enables the measurement of multiple phenotypic and functional assays from the same cells in CellCage™ enclosures. (A) Tens of thousands of cells are mixed with hydrogel precursor and loaded on an 8-lane flow cell. Cell positions are identified and CellCage™ enclosures (CCEs) automatically generated around cells with light-guided polymerization. Bio-compatible CCEs can be formed around single cells, multiple cells, or cells with objects (e.g., cytokine beads). CCEs are permeable to reagents enabling long-term culturing and a variety of imaging-based, longitudinal phenotypic and functional assays to be performed on the same cells (e.g., small molecules, immunofluorescent antibodies). Cells are lysed within CCEs to generate cDNA for downstream library prep and sequencing off the instrument. **(B)** Imaging-based phenotypic analysis enabled for single CCEs across flow cell lane. Cells within the same CCE can be processed for sequencing readouts and linked to imaging data. **(C)** Reagents can be diffused through semi-permeable CCE walls. This feature can be used to deliver nutrients, small molecules, and antibodies to all CCEs at once, at any point of the experiment. **(D)** Step-by-step overview of how imaging data is linked to mRNA. Encapsulated cells are serially imaged across brightfield and fluorescence channels. In these representative images, 3 markers are detected on the same cells. After imaging data is collected, Cellanome's computer vision capabilities segment cells, barcodes. Cells are then lysed within CellCage™ enclosures for cDNA synthesis on the flow cell.

IMAGING-BASED DETECTION OF MULTIPLE SENESCENCE MARKERS

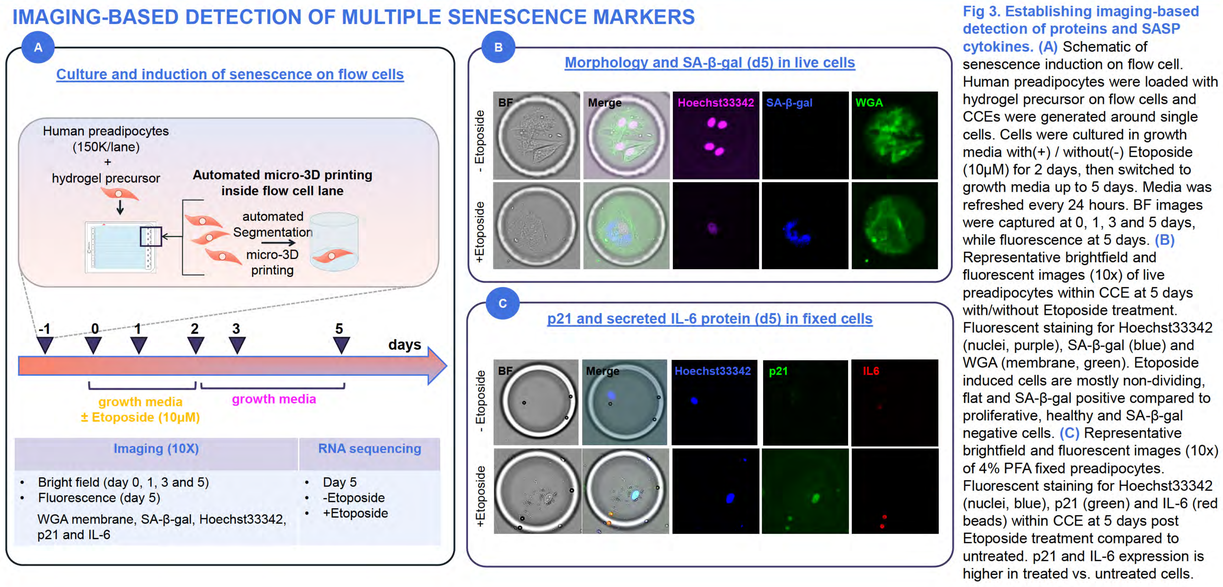


Fig 3. Establishing imaging-based detection of proteins and SASP cytokines. (A) Schematic of senescence induction on flow cell. Human preadipocytes were loaded with hydrogel precursor on flow cells and CCEs were generated around single cells. Cells were cultured in growth media with(+) / without(-) Etoposide (10μM) for 2 days, then switched to growth media up to 5 days. Media was refreshed every 24 hours. BF images were captured at 0, 1, 3 and 5 days, while fluorescence at 5 days. **(B)** Representative brightfield and fluorescent images (10x) of live preadipocytes within CCE at 5 days with/without Etoposide treatment. Fluorescent staining for Hoechst33342 (nuclei, purple), SA-β-gal (blue) and WGA (membrane, green). Etoposide induced cells are mostly non-dividing, flat and SA-β-gal positive compared to proliferative, healthy and SA-β-gal negative cells. **(C)** Representative brightfield and fluorescent images (10x) of 4% PFA fixed preadipocytes. Fluorescent staining for Hoechst33342 (nuclei, blue), p21 (green) and IL-6 (red beads) within CCE at 5 days post Etoposide treatment compared to untreated. p21 and IL-6 expression is higher in treated vs. untreated cells.

VALIDATING INDUCTION AND DETECTION OF SENESCENCE MOLECULAR SIGNATURES

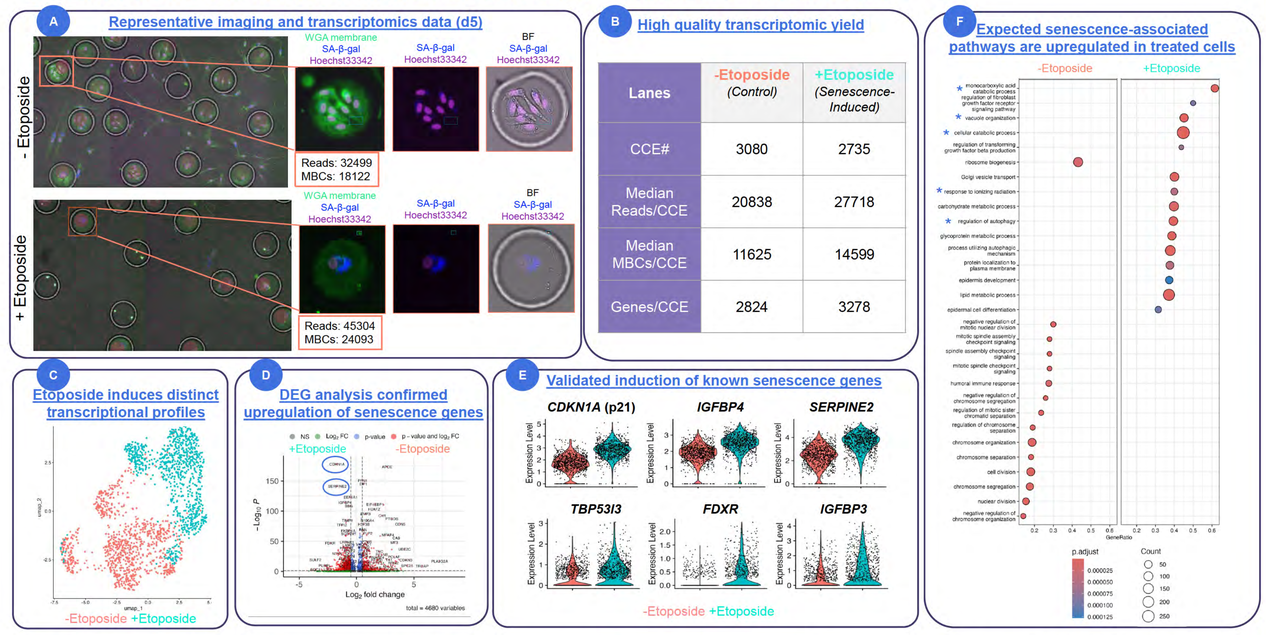
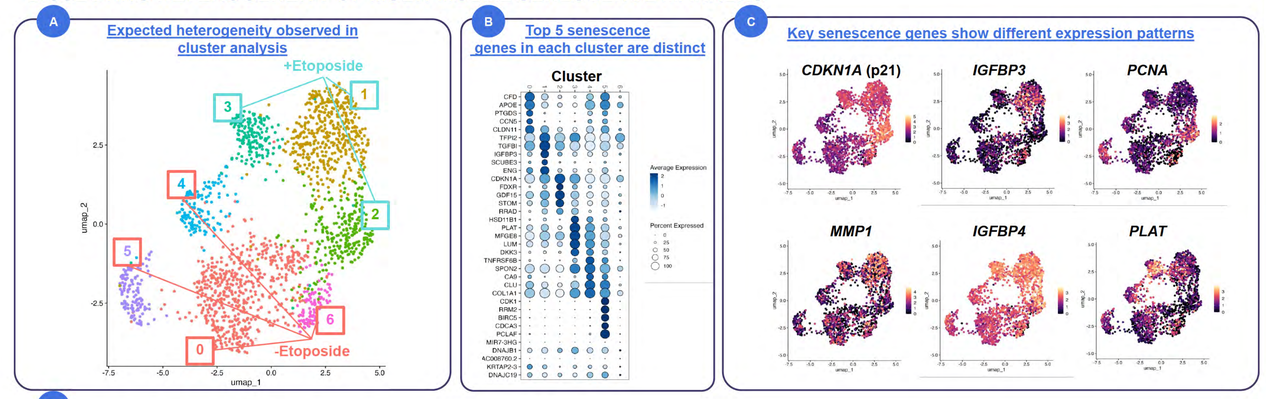


Fig 4. Converging transcriptomics to senescence markers detected by imaging with Cellanome's Technology. (A) Representative images of partial FOVs from ± Etoposide treated lanes showing CCEs overlapping molecular barcodes (light red). Insert zooms in on a single CCE and its associated number of reads and MBCs (Molecular Barcodes) (Hoechst33342 purple, SA-β-gal blue and WGA green). **(B)** RNA sequencing QC metrics. **(C)** Combined UMAP clustering for lanes ± Etoposide treatment. **(D)** Specific differential expression of known senescence genes between ± Etoposide treated lanes. **(E)** Expression levels of key senescence genes. **(F)** Gene set enrichment analysis showing selective enrichment or de-enrichment of senescence-associated pathways in Etoposide treated cells.

DISSECTING HETEROGENEITY OF MULTI-MODAL SENESCENCE MARKERS



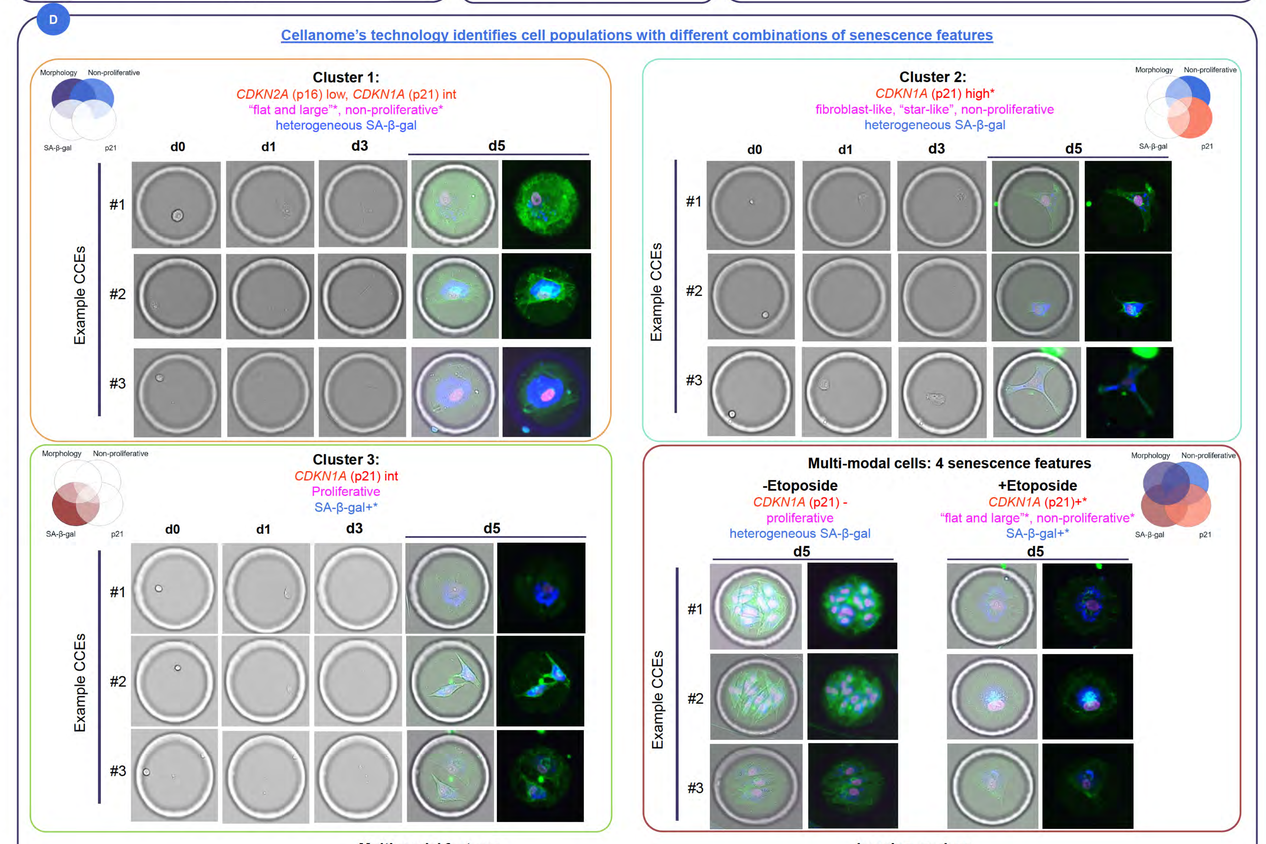


Fig 5. Identifying cells with multi-modal hallmarks of senescence by converging protein, transcriptomics, and morphology measurements to the same single cells. (A) Combined UMAP clustering for ± Etoposide treated cells. **(B)** Dot plot of top 5 differential expressed genes in each clusters. **(C)** Features plot showing selected senescence genes across clusters. **(D)** Representative BF and fluorescence images of CCEs over 0, 1, 3 and 5 days for + Etoposide treated preadipocytes across transcriptomic clusters (Cluster 1, 2 and 3). **Bottom right:** Representative images of cells with comprehensive co-expression of 4 multi-modal senescence markers (p21+, flat and large, non-proliferative, and SA-β-gal+) found across +Etoposide clusters, paired with representative images from –Etoposide cells. Fluorescent staining for Hoechst33342 (purple), SA-β-gal (blue) and WGA (green).

DISCUSSION AND FUTURE DIRECTIONS

We demonstrate that Cellanome's technology can be used to decipher cellular senescence and generate multi-modal dataset within the same individual cells. This includes imaging-based detection of larger morphology, with p21 expression, SA-β-gal activity, and / or secretion of SASP factors, any of which can be linked to transcriptomics. Our next steps are to 1) continue converging more senescence measurements from the same cells with single experiments, 2) validate this method in more senescence models, 3) develop quantification methods, and 4) apply multi-modal detection of senescence to experiments co-culturing both senescent and healthy cells. We believe this method will increase confidence in senescence studies by finding "true" senescent cells expressing multiple known markers, accelerate atlas initiatives seeking to identify tissue-specific senescence markers, understand specificity of senolytic drugs and unlock access to novel questions, including kinetics of senescence acquisition and whether senescent cells can transmit this phenotype to healthy cells.

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