Extracellular ATP Induced Senescence in Human Lung Cancer A549 Cells

Ryan Ward [1], Haiyun Zhang [2, 3, 4], Arabella Hunt [1], Nora Anderson [1], Xiaozhuo Chen [2, 5]

1. Honors Tutorial College, 2. Edison Biotechnology Institute, 3. Department of Biological Sciences, 4. Interdisciplinary Graduate Program in Molecular and Cellular Biology, 5. Department of Biomedical Sciences Heritage College of Osteopathic Medicine. Ohio University, Athens, OH 45701 USA

Introduction

- In response to stressful conditions or damage, cells can undergo a process known as senescence. In healthy tissue, senescence is an irreversible cellular state characterized by an enlarged morphology, a complete arrest of the cell cycle, and remodeling of the secretory phenotype[1].
- The Senescence Associated Secretory Phenotype (SASP) contains high levels of growth factors, cytokines, and proteases which promote proliferation, avoiding cell death, and angiogenesis in the surrounding cells[2].
- Due to its antiproliferative nature, senescence was seen for a long time as an anticancer mechanism that should be targeted for induction by chemotherapeutic strategies, but recent studies have shown that senescence in tumor cells can promote cancer progression[3].
- Cancer cells can senescence in response to chemotherapy which allows them to have heightened drug resistance capabilities to survive the treatment[4].
- Unlike in healthy tissue, senescence in cancer cells is a reversible process. When cancer cells come out of a senescent state, they will have heightened stem cell characteristics while retaining parts of the SASP which provides them with increased proliferative potentials and other hallmark capabilities[4].
- Senescent cells have recently been named an emerging hallmark of cancer[3].
- We recently found extracellular ATP (eATP) to induce Cancer Stem Cell (CSC) formation in a similar way to TGF-β, a known CSC inducer and cytokine involved in senescence inducing pathways[4,5].
- Based off our previous data, I hypothesized that eATP induces a reversible senescence like state as a precursor to the CSC induction in lung cancer.
- Senescent cells can be identified by selectively staining for senescence associated β-galactosidase activity through increased expression of the GLB1 gene[6].

Materials and Methods

- **ATP:** Purchased from Sigma-Aldrich.
- **Cell lines:** Human non-small cell lung cancer (NSCLC) cell lines A549 were purchased from ATCC.
- **Culture Media:** Cells were cultured in F-12K media purchased from Thermo Fisher supplemented with 10% FBS.
- **RNA sequencing:** Used to determine the gene expression profile of eATP and TGF-β treatments.
- **Senescence Associated Beta-Galactosidase Activity Chromogenic Assay:** Used to selectively stain for senescent cells for detection under a bright field microscope[6].
- **CellEvent™ Senescence Green Flow Cytometry Assay:** Used to selectively stain for senescent cells for detection with flow cytometry, protocol was performed according to manufacturer's instructions.

Results

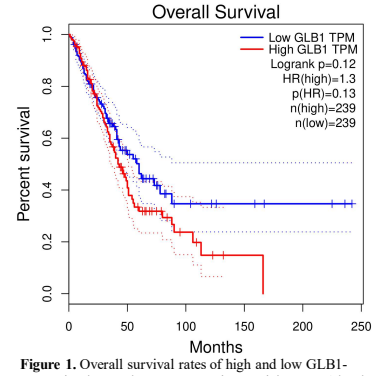

Figure 1. Overall survival rates of high and low GLB1-expressing human lung cancer patients. Higher STC1 levels were associated with lower survival rate. Data was generated from GEPIA (http://gepia.cancer-pku.cn/index.html).

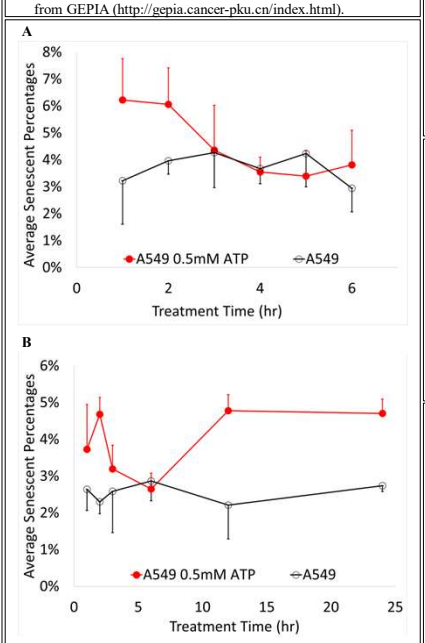

Figure 4. Percentages A549 cells staining positive using the senescence associated β-galactosidase activity chromogenic assay over an eATP treatment time course of **A.** 6 hours or **B.** 24 hours compared to an untreated control counted over a representative sample.

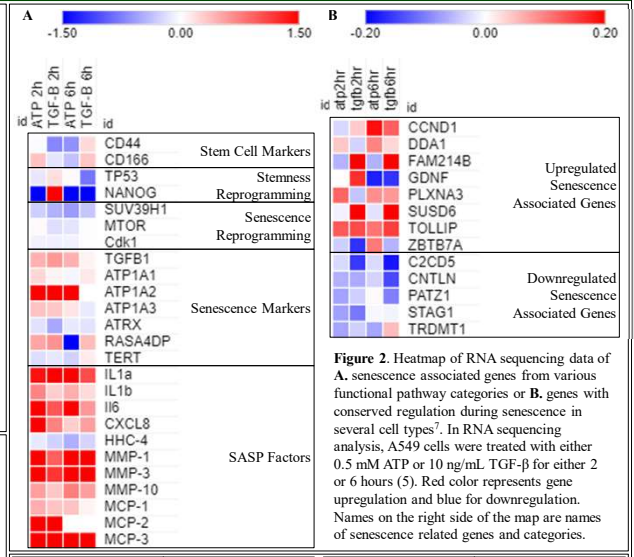

Figure 2. Heatmap of RNA sequencing data of **A.** senescence associated genes from various functional pathway categories or **B.** genes with conserved regulation during senescence in several cell types[7]. In RNA sequencing analysis, A549 cells were treated with either 0.5 mM ATP or 10 ng/mL TGF-β for either 2 or 6 hours (5). Red color represents gene upregulation and blue for downregulation. Names on the right side of the map are names of senescence related genes and categories.

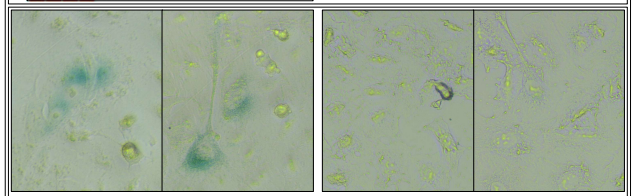

Figure 3. eATP induces senescence in A549 cells. Human NSCLC A549 cells were treated with (left) or without (right) 0.5 mM ATP, the concentration of eATP found in TME for 2 hours. Cells were using the senescence associated β-galactosidase activity chromogenic assay. Senescent cells stain blue. Senescent cells also generally appear larger than normal cancer cells.

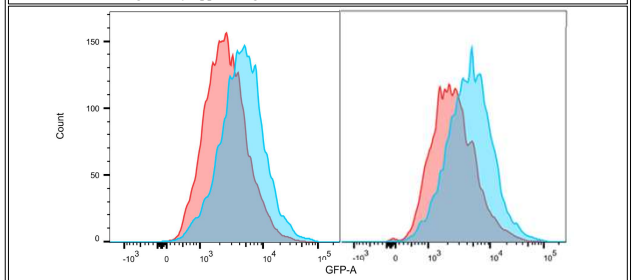

Figure 5. Histograms showing counts of cells over a logarithmic fluoresce scale of from the CellEvent™ Senescence Green Flow Cytometry Assay. A549 cells were treated with (blue) or without (red) 0.5mM ATP for 1 (left) and 24 (right) hours.

Conclusions and Future Directions

- High expression of GLB1, a marker of senescence, in tumor cells is a predictor of lower survival chances in lung cancer patients.
- Many senescence associated genes have upregulated transcription after eATP or TGF-β treatment implying the induction of a senescent like state.
- eATP induces an increase in senescence associated β-galactosidase activity showing a senescence like state after only 1-2 hours of treatment which reverses after 6 hours.
- The increase in senescence associated β-galactosidase activity was also confirmed by flow cytometry.
- This data is consistent with the CSC induction previously described in which CSC marker expression reaches its peak at 6 hours[5].
- The next phase of this project will involve reducing the expression of specific genes using siRNA to observe the effect this has on eATP senescence induction.
- Targeted genes will include TGFB1 (TGF-β), CDKN2A (p16), and targets of the transcription factors Oct4 and NANOG which are essential for stemness reprogramming[4].
- We will also look into epigenetic alterations made in response to eATP treatment as non-mutational epigenetic reprogramming is both an emerging hallmark of cancer and a central regulatory mechanism of senescence[1,3].

References

1. Hernandez-Segura, A., Nehme, J., & Demaria, M. (2018). Hallmarks of Cellular Senescence. Trends in cell biology, 28(6), 436–453.
2. Coppé, J. P., Desprez, P. Y., Krtolica, A., & Campisi, J. (2010). The senescence-associated secretory phenotype: the dark side of tumor suppression. Annual review of pathology, 5, 99–118.
3. Hanahan D. (2022). Hallmarks of Cancer: New Dimensions. Cancer discovery, 12(1), 31–46.
4. Zhang, D. Y., Monteiro, M. J., Liu, J. P., & Gu, W. Y. (2021). Mechanisms of cancer stem cell senescence: Current understanding and future perspectives. Clinical and experimental pharmacology & physiology, 48(9), 1185–1202.
5. Song, J., Qian, Y., Evers, M., Nielsen, C. M., & Chen, X. (2022). Cancer Stem Cell Formation Induced and Regulated by Extracellular ATP and Stanniocalcin-1 in Human Lung Cancer Cells and Tumors. International journal of molecular sciences, 23(23), 14770.
6. Debacq-Chainiaux, F., Erusalimsky, J. D., Campisi, J., & Toussaint, O. (2009). Protocols to detect senescence-associated beta-galactosidase (SA-betagal) activity, a biomarker of senescent cells in culture and in vivo. Nature protocols, 4(12), 1798–1806. https://doi.org/10.1038/nprot.2009.191
7. Hernandez-Segura, A., de Jong, T. V., Melov, S., Guryev, V., Campisi, J., & Demaria, M. (2017). Unmasking Transcriptional Heterogeneity in Senescent Cells. Current biology : CB, 27(17), 2652–2660.e4.

Acknowledgments

This project was partially supported by, the John J. Kopchick MCB/TBS Undergraduate Student Support Fund, the Provost Undergraduate Research Fund, as well as a National Institute of Health (NIH) R15 CA242177-01 Grant to Xiaozhuo Chen