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Analyzing Senolytic Compounds induced Cellular Senescence Response using a Triple Quadrupole Mass Spectrometer

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1. Novel Aspect

Using an LC-MS/MS method to compare the chemical profile in the cell environments of young, senescent, and senolytics-dosed senescent cells

2. Introduction

Cellular senescence occurs when cells undergo various stresses. Senescent cells and their secretions can cause tumor growths and chronic inflammation that triggers age-related diseases. Senolytic compounds are pharmaceuticals that selectively eliminate senescent cells through various mechanisms. While it is challenging to identify the senolytic mechanism, it is crucial for the development of senolytic drugs.

Comprehensive analysis and comparison of the media environments from young cells, senescent cells, and senescent cells dosed with different senolytics can contribute toward solving senolytic mechanisms. Understanding the effects of senolytic compounds by analyzing the intake and secretion of the senescent cells can make the drug discovery process more efficient and effective.

3. Methods

Young, senescence, and senolytic (753b or EF24)-dosed senescent cells were incubated in WI38 cell culture media for 72 hours. Cell culture media was collected at 10 different growth time points. Prior to analysis, media samples were subjected to acetonitrile for protein crash then further diluted with water. A 17-minute liquid chromatography triple quadrupole mass spectrometry method was developed on a Shimadzu LCMS-8060 for senescent cell and senolytic-added senescent cell culture media analysis. Mobile phase for the chromatography were water and acetonitrile with 0.1% formic acid. One hundred and forty-four cell culture related compounds including amino acids, nucleic acids, metabolites, sugars, and vitamins were simultaneously analyzed using both positive and negative modes of the ESI source. Data were collected in triplicates.

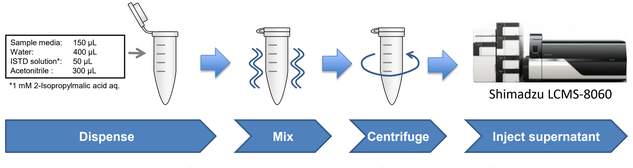

Figure 1. Sample preparation procedure for cell culture profiling analysis

Table 1. LCMS Analytical Conditions

LC Conditions	[LC-30 series]
Mobile Phase A	: Water containing 0.1% formic acid
Mobile Phase B	: Acetonitrile containing 0.1% formic acid
Chromatography Gradient	: 0% B -> 95% B
Total Run Time	: 17 minutes
Column	: Cell Culture Profiling Column (150mm x 2.1mm, 3µm)
Flow Rate	: 0.35 mL/min
Column Oven Temperature	: 40℃
Injection Volume	: 1 uL
MS Conditions	[LCMS-8060]
Ionization Mode	: ESI +/-
Acquisition Mode	: MRM
Nebulizing Gas Flow	: 3.0 L/min
Heating Gas Flow	: 10.0 L/min
Interface Temperature	: 300 ℃
DL Temperature	: 250 ℃
Heat Block Temperature	: 400 ℃
Drying Gas Flow	: 10.0 L/min

4. Results

Thirteen compounds exhibited trends that distinguished at least one experimental group from the others in the 72 hours growth period are shown in Figures 2 and 3. The differences observed in the cell environment reflect the differences of the activated pathways and mechanisms between the young, senescent, and senolytic-dosed senescent cells.

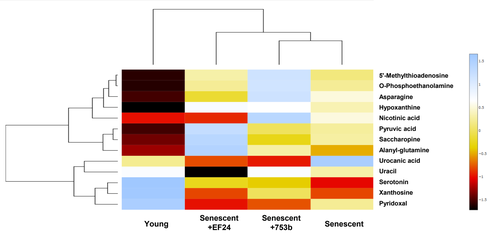

Figure 2. Heat map of differentially expressed analytes in the cell culture environment of young, senescent, and senolytic-added senescent cells at culture hour 72.

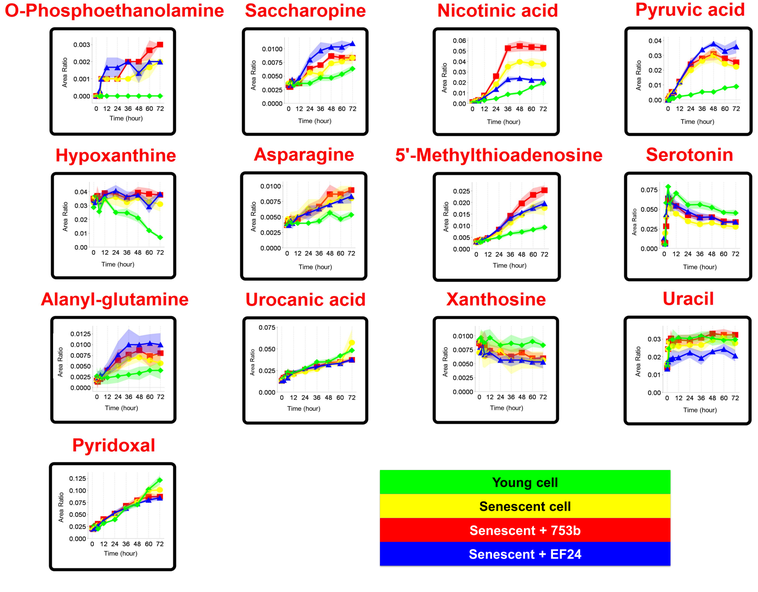

Figure 3. Relative concentration trend graphs of differentiating compounds in the cell culture environment of young (green), senescent (yellow), and senolytic-added senescent cells (red – 753b and blue – EF24) throughout the entire culture period (72 hours).

O-phosphoethanolamine is an important intermediate in the biosynthesis of phosphatidylethanolamine, a type of phospholipid that is found in cell membranes. It is also involved in the regulation of lipid metabolism and has been shown to have antitumor and antiviral activities. In our study, senescent cells can secret O-phosphoethanolamine while young cells cannot.

Saccharopine is an intermediate molecule that plays a crucial role in the metabolism of the amino acids lysine and methionine. The breakdown of saccharopine into α-ketoadipic acid and L-arginine is catalyzed by the enzyme saccharopine dehydrogenase (SDH). SDH activity decreases in the diaphragm muscle of older rats compared to young rats,[1] which may explain the increased secretion of saccharopine from senescent cells. EF24 can further enhance the secretion of saccharopine.

Nicotinic acid has been shown to regulate the cellular senescence through activating the sirtuin pathway. Activation of the sirtuin pathway has been linked to various health benefits, including improved metabolic function, reduced inflammation, and protection against age-related diseases such as cancer, Alzheimer's disease, and cardiovascular disease. Additionally, nicotinic acid also has anti-inflammatory effects, which may play a role in regulating senescence. Nicotinic acid can be synthesized from the amino acid tryptophan through the kynurenine pathway. Studies have shown that the expression of tryptophan 2,3-dioxygenase (TDO), the enzyme responsible for the initial step in the kynurenine pathway, is upregulated in senescent cells.[2] In addition, activation of the kynurenine pathway has been linked to the production of reactive oxygen species (ROS), which contributes to the development of cellular senescence. 753b and EF24 exhibited distinct roles in regulation of nicotinic acid secretion.

Pyruvic acid provides a source of energy for cells. Senescent cells have reduced metabolic activity thus rely on pyruvic acid more as an alternative energy source. In addition to its role in energy production, pyruvic acid has been shown to have antioxidant and anti-inflammatory properties, which can help protect senescent cells from oxidative stress and inflammation. Pyruvic acid has been reported to increase the expression of antioxidant enzymes and decrease the production of reactive oxygen species (ROS) in senescent cells.[3] This can reduce the accumulation of oxidative damage and delay the onset of senescence. In our study, much more pyruvic acid was detected in the extracellular media of senescent cells compared with young cells, which suggests senescent cells may generate more pyruvic acid for producing energy and removing ROS to maintain their cellular activity. EF24 can further enhance the secretion of pyruvic acid after a longer period (>24 h).

Hypoxanthine is a naturally occurring purine base that is a precursor to both adenine and guanine. Dividing cells require more hypoxanthine because it is an essential component of nucleotides, so young cells consume more hypoxanthine while senescent cells do not take up hypoxanthine. The cessation of hypoxanthine consumption can be used as an important indicator of cellular senescence.

Asparagine is involved in protein synthesis and is important in the formation of the peptide bonds. It is also involved in the synthesis of glycoproteins. Asparagine is also a precursor to other amino acids such as aspartic acid, lysine, methionine, and threonine. Compared with young cells, our result showed that senescent cells secreted more asparagine.

5'-Methylthioadenosine (MTA) is a potent inhibitor of S-adenosylmethionine (SAM)-dependent methyltransferases. By inhibiting these enzymes, MTA can affect a wide range of cellular processes, including gene expression, protein synthesis, and epigenetic regulation. Once produced, MTA can be exported from the cell and released into the extracellular space, where it can potentially interact with other cells and tissues. The secretion of MTA by cells has been observed in various biological contexts, including inflammation, cancer, and tissue injury. In these settings, MTA can act as a signaling molecule that can affect the behavior of nearby cells. MTA is a powerful inhibitor of inflammation and has anti-apoptotic effect for normal cells.[4,5] In our study, senescent cells secret more MTA, which may partially explain the reason that senescent cells are resistant to immune clearance.

Serotonin is a chemical messenger. The storage, release, and reuptake of serotonin is an important mechanism for regulating its concentration in the synapse. It is remarkable to observe the WI38 lung fibroblast cells secret serotonin quickly when changed to a fresh medium and then reuptake it gradually. Compared with young cells, the result showed that senescent cells secret less serotonin.

4. Conclusion

This rapid and multicomponent cell culture analysis method enables scientists to analyze the environment as senescent cell and senolytic-dosed senescent cells grow. Comparing the chemical profile in the cell environments can be a potential strategy in understanding the mechanisms of the senolytic drugs. This information may also be valuable in predicting the toxicity and side effects of the new senolytic drugs.

5. Reference

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