Analysis of the Role of ES1 Protein on Methylglyoxal (MGO) and Advanced Glycation End Products (AGEs)

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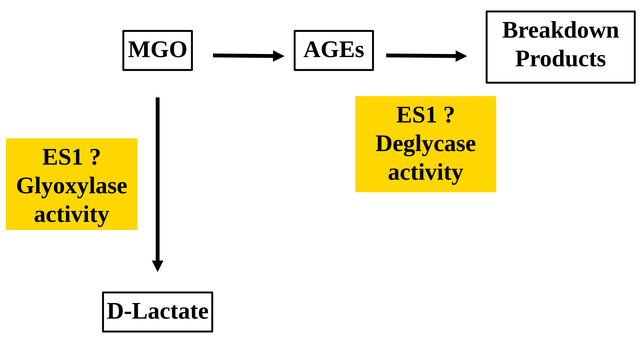
Introduction

- Methylglyoxal (MGO) is a dicarbonyl metabolite primarily derived from the spontaneous degradation of glycolysis intermediates.
- MGO is a major contributor to glycation, a nonenzymatic glycosylation process, which leads to the formation of advanced glycation end products (AGEs).
- AGEs are demonstrated to cause irreversible damage to proteins, disrupting their structure and function, which leads to further disease.
- As the heart ages and in various pathologies such as hypertrophy and infarction, the heart relies more on glycolysis for energy production. This increase in glycolysis increases MGO formation which induces dicarbonyl stress, leading to elevated AGE levels.
- MGO should be removed by the dicarbonyl metabolic system, yet the mitochondrial mechanisms involved remain poorly understood. Previous studies suggest that the mitochondrial protein ES1 might exhibit either glyoxylase or deglycase activity, aiding in MGO degradation or AGE degradation.
- If the mitochondrial dicarbonyl metabolic system and the role of ES1 is fully understood, it can be used to help develop strategies for preventing AGE formation, thereby promoting heart health in aging and various pathologies.



Hypothesis

- AC16, a cell line derived from primary adult human ventricular tissue, overexpressing ES1 will exhibit increased survival when treated with MGO compared to control cells.
- AC16 cells overexpressing ES1 will exhibit reduced AGE levels when treated with MGO compared to control cells.

ES1 Overexpression Confirmation

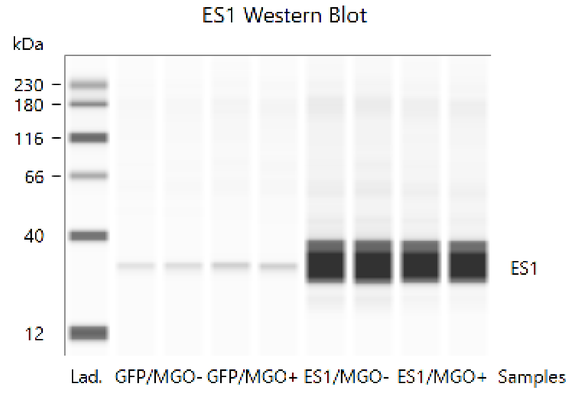


Figure 1: Increased Western blot signal indicates successful ES1 protein overexpression in cultured AC16 cells with adenovirus-mediated ES1 overexpression.

MTT Assay

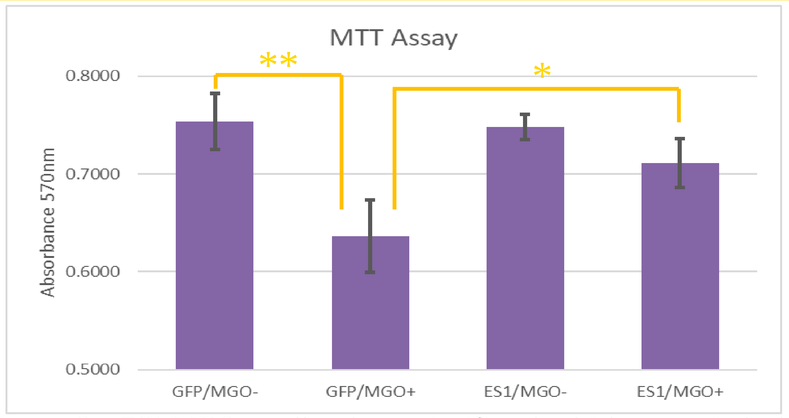


Figure 2: ES1/MGO+ cells show significantly higher survival than GFP/MGO+ cells (*p < 0.05, n=5 per group, Student's T-test) while showing similar survivability compared to ES1/MGO- cells (p > 0.05, n=5 per group, Student's T-test). *p < 0.05; **p < 0.01

AGE ELISA

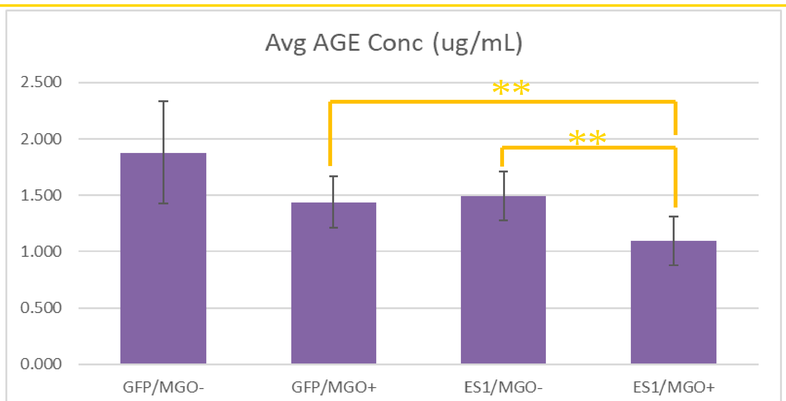
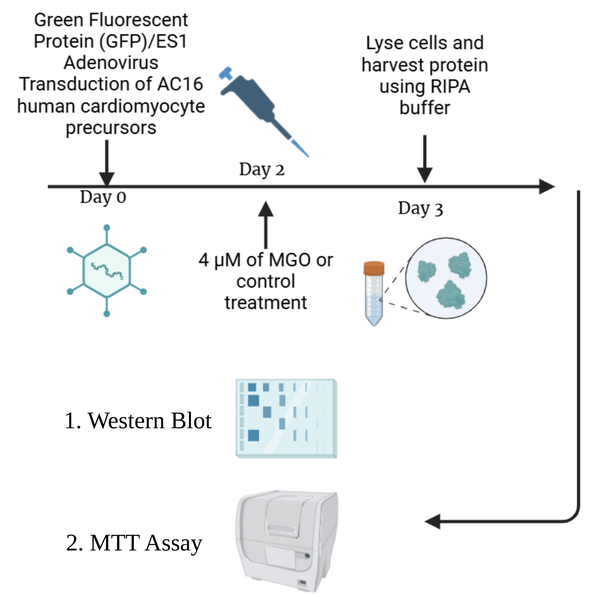


Figure 3: ES1/MGO+ cells show significantly less AGE concentration than GFP/MGO+ and ES1/MGO- cells (**p < 0.01, n=6 per group, Student's T-test).

Methods



Discussion and Future Directions

- We first confirmed ES1 overexpression by performing a Western blot, followed by an MTT assay to assess cell viability, and an ELISA to measure AGE concentration.
- Our results show that cells overexpressing ES1 have better survival and reduced AGE concentrations in the presence of MGO, indicating that ES1 may help breakdown MGO and AGEs.
- In future studies, we will measure cell viability and AGE concentrations for cells exposed to higher concentrations of MGO for a longer period of time.

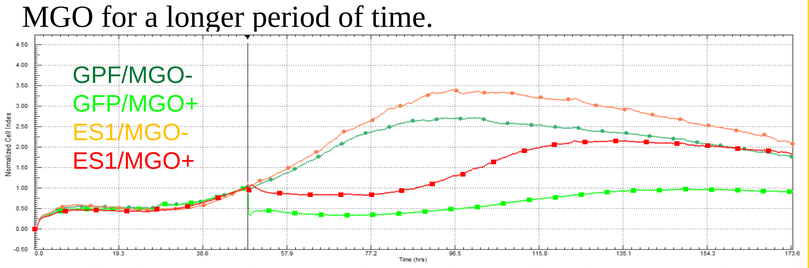


Figure 4: The start of a study for our future directions measuring cell viability in the presence of 400 uM MGO for 7 days using xCELLigence.

References

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2. Nigro, C., Leone, A., Fiory, F., Prevenzano, I., Nicolò, A., Mirra, P., Beguinot, F., & Miele, C. (2019). Dicarbonyl stress at the crossroads of healthy and unhealthy aging. Cells, 8(7), 749. https://doi.org/10.3390/cells8070749