Functional characterization of nuclear receptor NR1D1 on investigation of brain metastasis

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Abstract

Brain metastasis is a fatal recurrence of advanced cancer that affects 20% of cancer patients. Capturing comprehensive brain metastasis landscape is critical to the establishment of sufficient and effective anti-tumor strategies. Ongoing research efforts in our team aim to better understand the biology of tumor cells in human brain metastasis. Preliminary data, generated by single cell sequencing of human brain metastases highlighted a potential role of the nuclear receptor NR1D1. Indeed, NR1D1 activity seems to be correlated with proliferation, immune evasion, and metabolism of brain metastatic tumor cells. The activity of this nuclear receptor can be controlled by its ligand Hemin (HEME) and is critical in retaining control of the circadian rhythm. Our aim is to investigate the functional properties of NR1D1 itself through breast cancer cell lines. Breast cancer cell lines, SUM-190, MDA-MB-231 and JIMT1 were used to study NR1D1 function and undergone multiple treatments with conditions that consisted of exposing HEME and a synthetic agonist in various incubation times while adjusting media with no FBS, essentially starving the cells from creating their own HEME. In publicly available data sets it was found that low NR1D1 expression correlated with increase of Triradylglcerols metabolism and high NR1D1 expression with increase of amino acids and peptide metabolism. In addition, our data suggests that NR1D1 activation leads to cell morphology changes and future experiments will determine whether NR1D1 influences tumor cell proliferation and immune invasion. This will assist in determining discrete phenotypes of brain metastatic tumor cells and new approaches for treatment.

Background

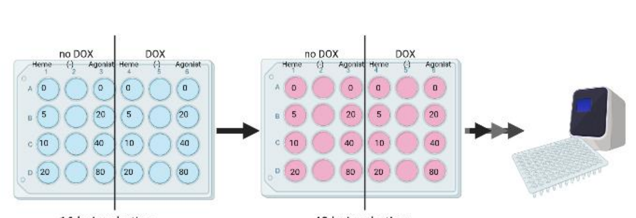
Brain metastasis is a common brain tumor and occurs upon the survival of circulating tumor cells passing the protective blood brain barrier, presenting a considerable challenge for effective treatment. It is unknown what biological mechanisms cause the successful establishment of human brain metastasis, however our lab's preliminary data from single cell sequencing showed evidence of a potential role of the transcription factor NR1D1 in brain metastasis.

This work focuses on understanding the role of NR1D1 receptor in breast cancer and its functional properties. NR1D1 belongs to the nuclear receptor family and plays the role in regulating the circadian rhythm. Due to preliminary data, it seems that NR1D1 controls genes involved in cancer functionality such as metabolism, proliferation, and immune invasion. These genes include CLOCK and ARTNL, an indicator of the circadian rhythm, and MIF, macrophage inhibitory factors, which inhibits the immune cells from attacking cancer cells thus prolonging the survival of tumor cells.

NR1D1 activity is controlled by Heme, a ligand that has a significant role in oxygen transport and has a promising role in tumor cell metabolism that can assist in cancer survival and proliferation. Breast cancer cells lines, including SUM190, JIMT1, and MDA-MD-231, were used to undergo treatments in various Heme and synthetic agonist concentrations.

Methods

- Manipulated the *in vitro* conditions of breast cancer cell lines, JIMT1, MDA-MD-231, and SUM190, by adding increasing Heme and Agonist concentrations.
- Cultured separate JIMT1 cell lines with SA, Heme, and Agonist and added HLA-ABC Monoclonal Antibody that show HLA presence within the membrane.
- Stained JIMT1 cell lines with SA, Heme, and Agonist stained with NucgreenTM dead 488 for percentage of cell death.
- With the use of public metabolic databases in finding low and high NR1D1 metabolisms, data was compiled.



Results

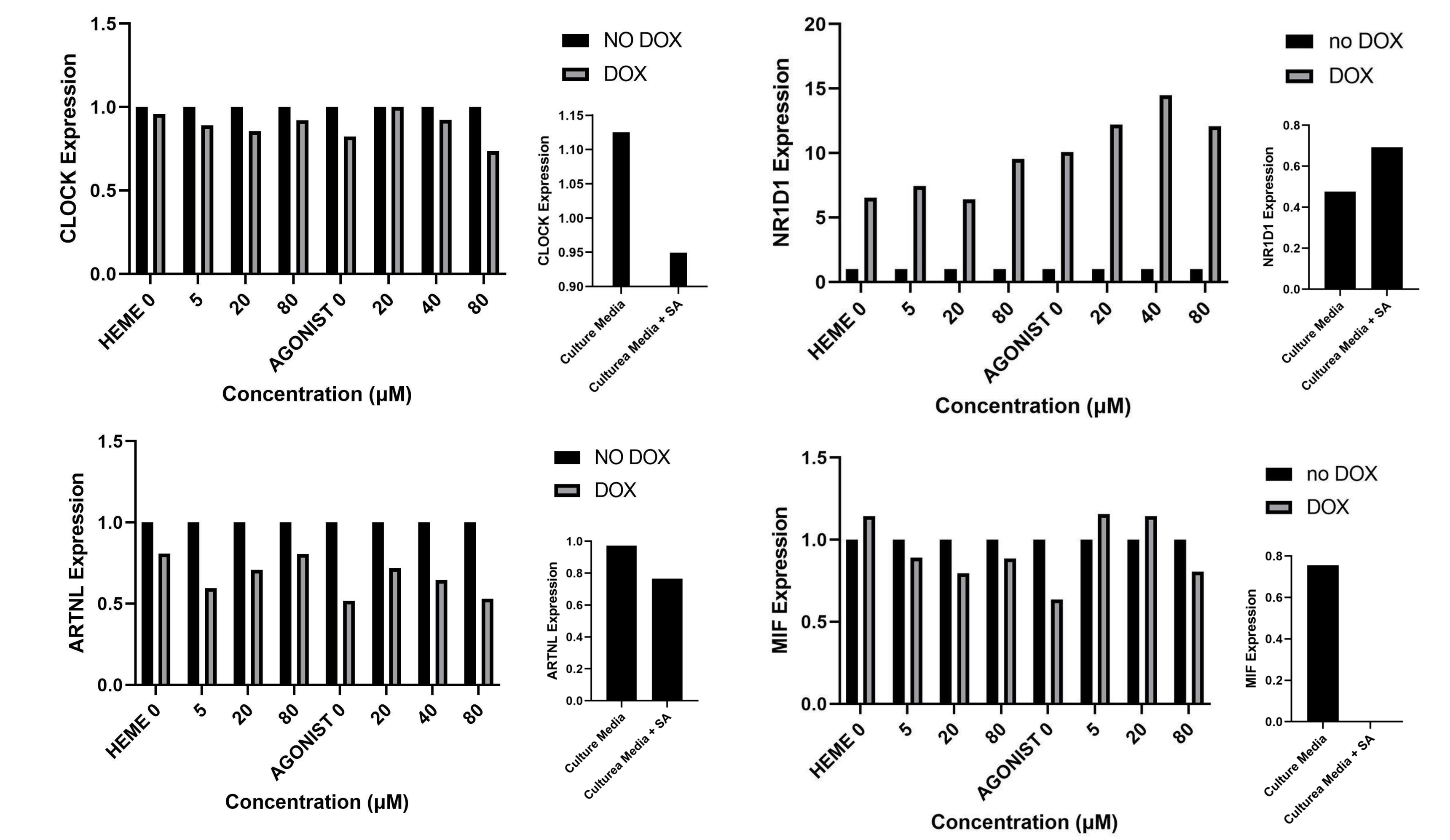


Figure 1. MDA-MD-231 analysis of NR1D1, CLOCK, ARTNL, and MIF gene expression after being in controlled conditions of Heme and Agonist of with and without doxycycline (DOX).

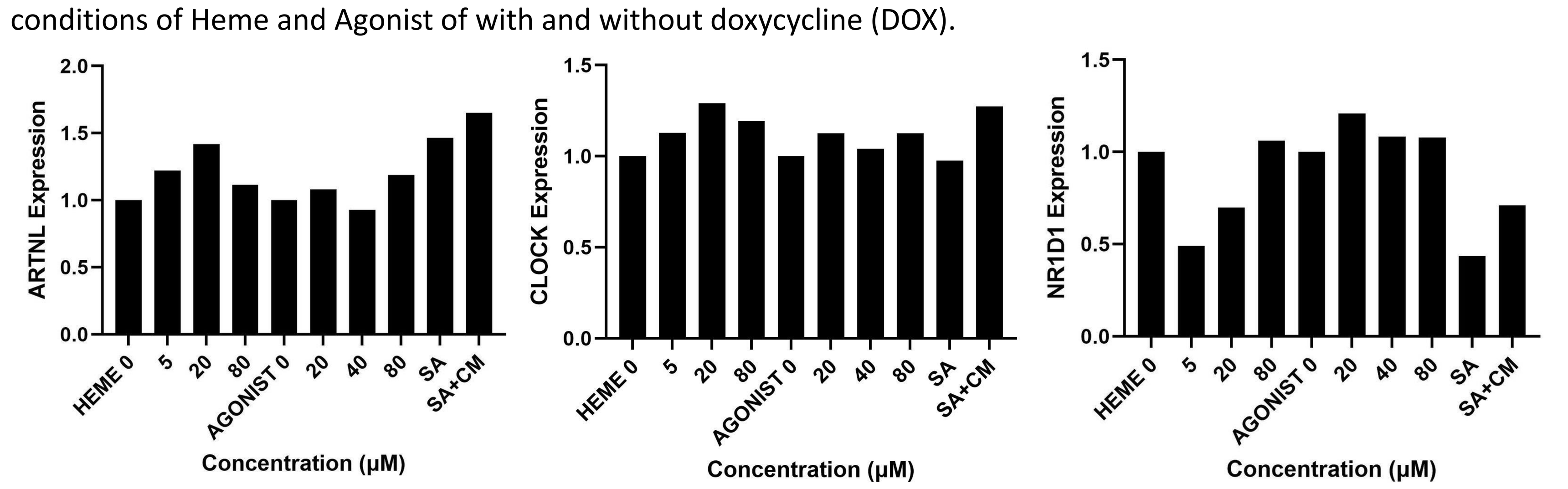


Figure 2. JIMT1 was treated under the increasing concentrations of Heme and Agonist; NR1D1, CLOCK, and ARTNL gene expression were recorded.

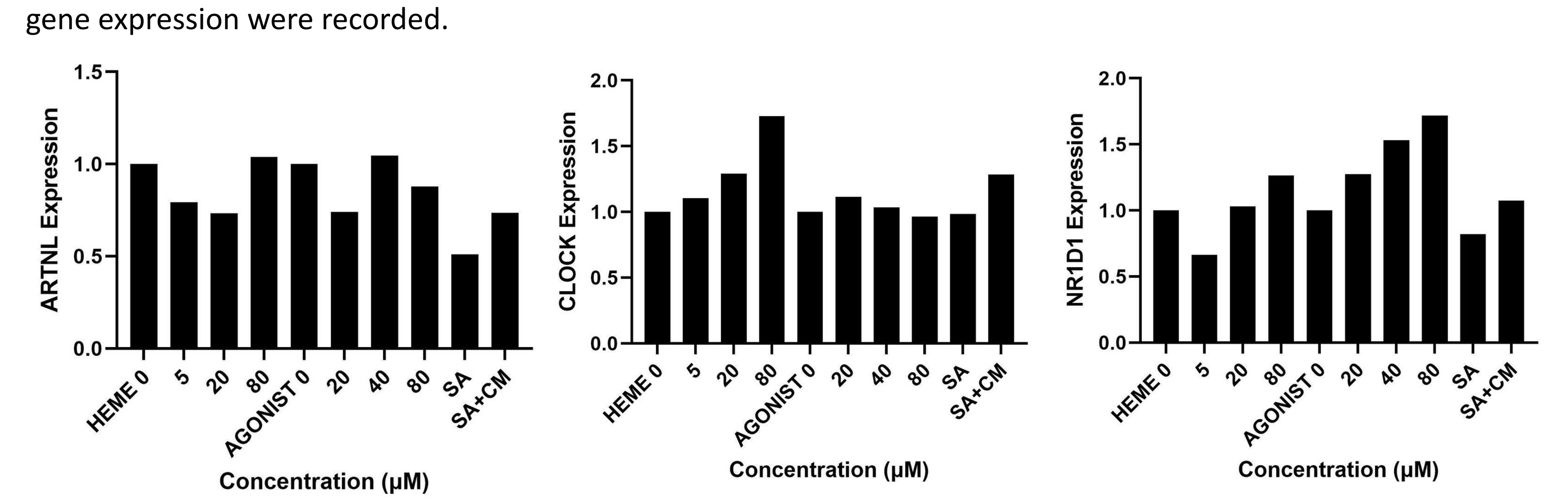


Figure 3. SUM190 gene expression of NR1D1, CLOCK, and ARTNL under treatments of increasing Heme and Agonist.

Figures 1, 2, and 3 overall demonstrate NR1D1 expression high in the concentrations of Heme and Agonist, with ARTNL, CLOCK, and MIF being expressed relatively below the normalized condition.

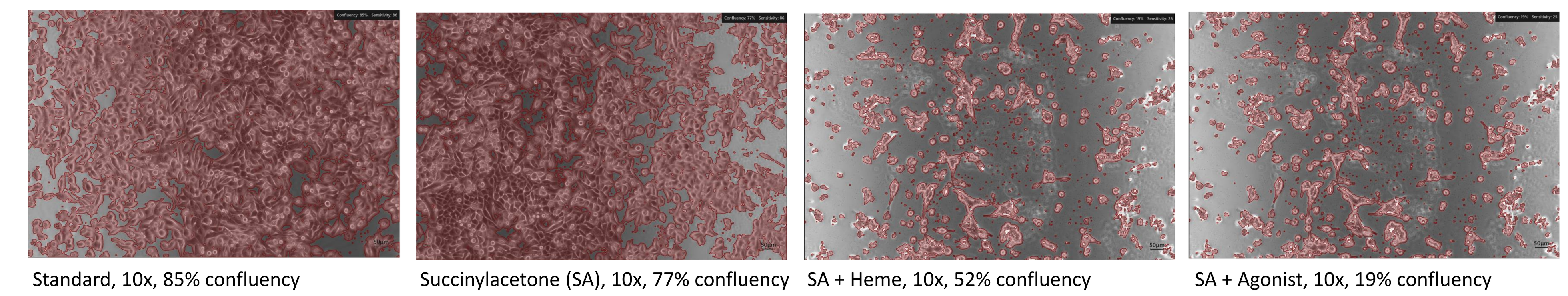


Figure 4. JIMT1 cells under 48-hour incubation. Confluency decreases as the presence of SA, heme, and agonist are introduced.

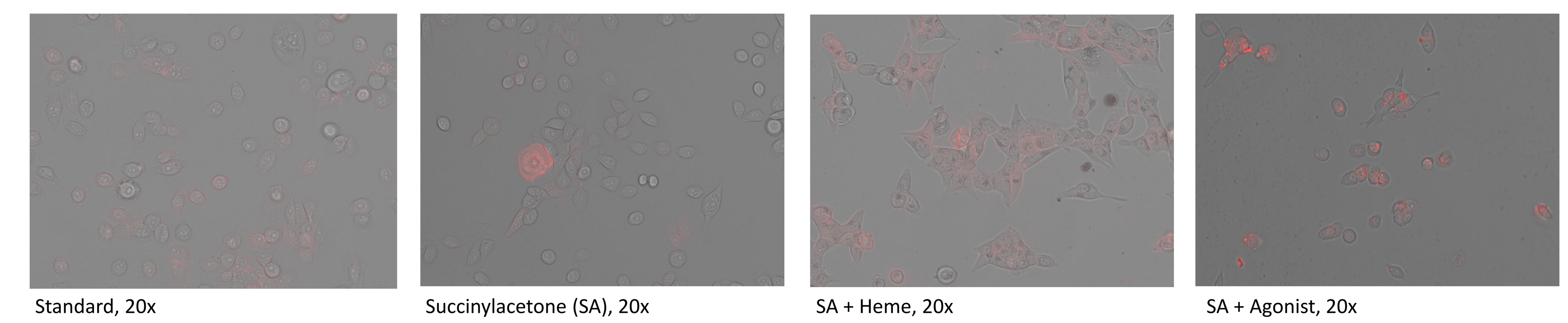


Figure 5. 20x overlay of JIMT-1 cells upon 48-hour incubation. HLA-ABC Monoclonal Antibody was used to indicate presence of HLA around membrane under PE fluorescence. HLA synthesis increases when exposed to SA, Heme, and Agonist.

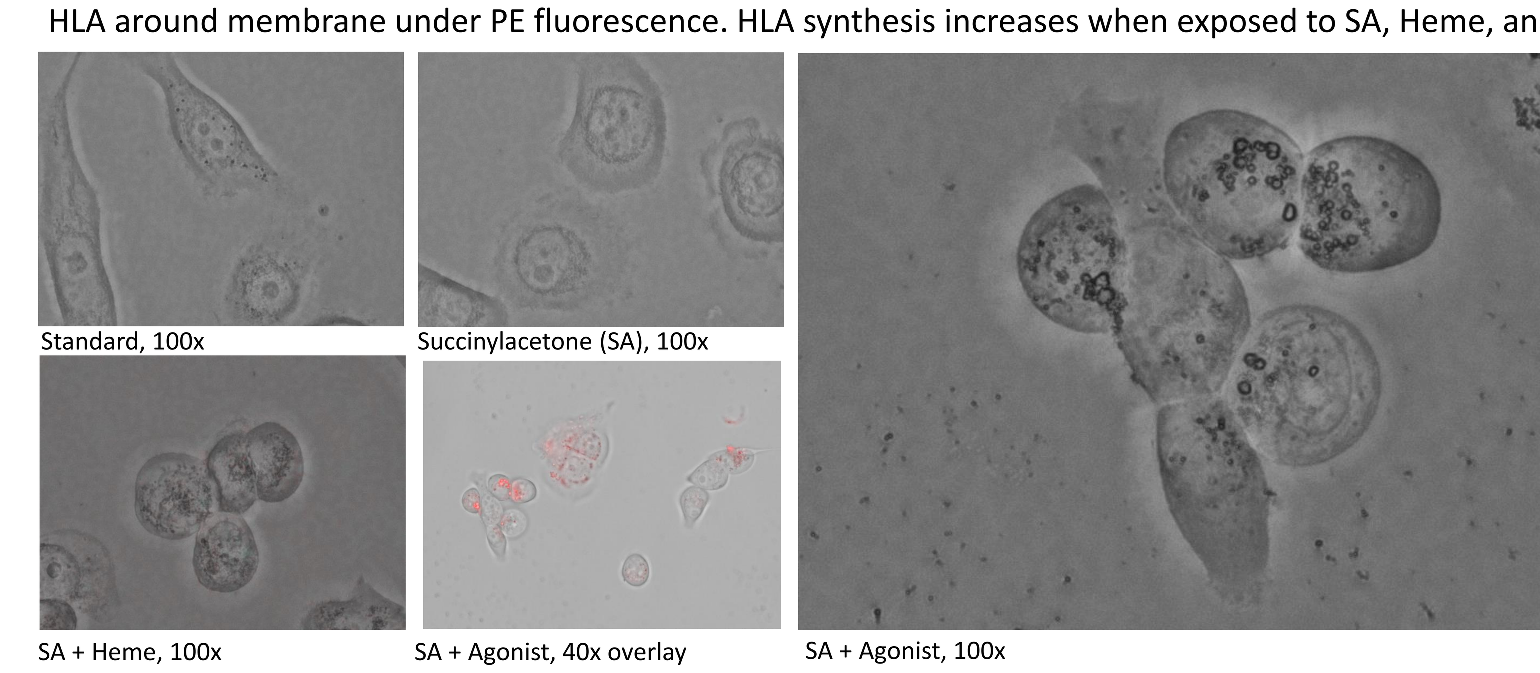


Figure 6. Cell morphology of JIMT-1 cells after 48-hour incubation of SA, Heme, and Agonist examined under 100x. SA + Agonist 40x overlay demonstrates the presence of HLA, coming from clusters surrounding the exterior of JIMT-1 treated with SA and Agonist.

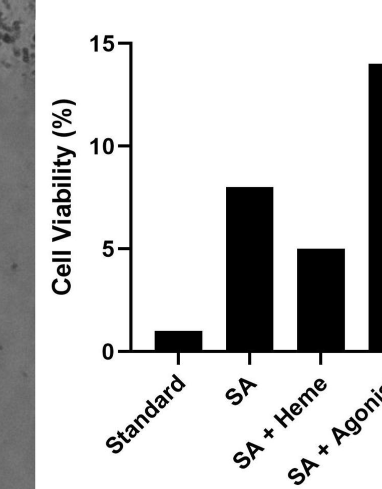


Figure 7. JIMT-1 cell viability via Nucgreen^{TM} dead 488 staining; percentage of cell death based on said conditions.

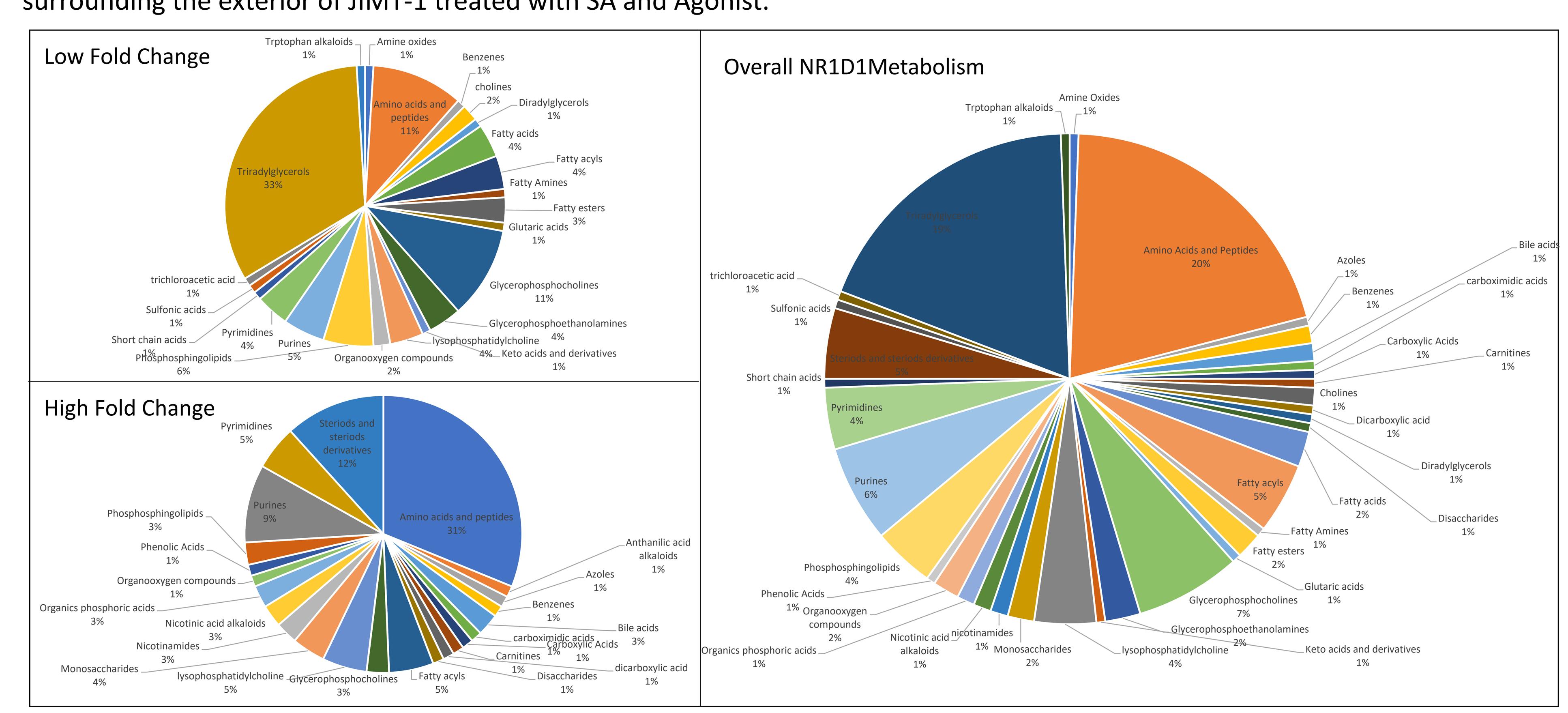
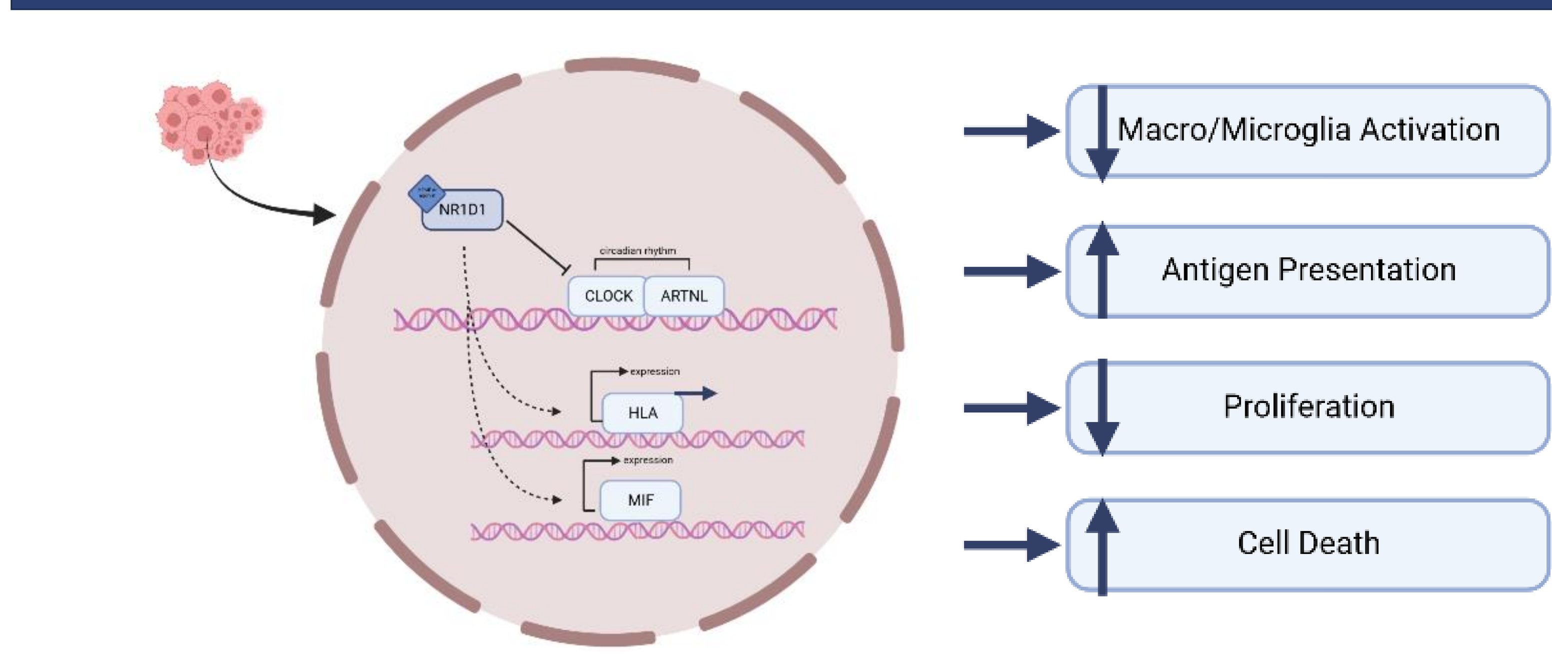


Figure 8. Data found through public databases demonstrates the distribution of NR1D1 metabolism. Low fold change shows that Triradyglycerols (33%) is synthesized with low expression of NR1D1, while High fold change, or high expression of NR1D1 synthesized more Amino Acids and Peptides (31%).

Conclusions & Future Directions



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In memory of Jocelyn Mercado and Marilou Mercado