# Chemotherapy Desensitization of Liver Cancer Cells with ATGL Overexpression

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## ABSTRACT

Adipose triglyceride lipase (ATGL) is a key enzyme in releasing fatty acids from triglycerides during lipolysis. ATGL can promote or inhibit cancer cell growth depending on the tissue. This experiment is testing the sensitization of chemotherapeutic agents in conjunction with ATGL overexpression in liver cancer. Three hepatocellular carcinoma cell lines (Hep3B, HepG2, and HUH7) were transduced and puromycin selected with two lentiviruses: one containing pLJM1 as a vector control and one containing hATGL to induce ATGL overexpression. Bodipy staining after oleate supplementation showed lower lipid droplet accumulation with ATGL overexpression for all cell types tested. Cell viability in standard and oleate supplemented conditions showed ATGL overexpression generally decreasing sensitivity to the FDA approved chemotherapeutic agent doxorubicin. The findings support the idea that the breakdown of triglycerides could be supporting the growth and viability of cancer cells. Future research on the mechanisms behind ATGL mediated drug resistance in relation to cancer therapy and oxidative stress could provide new targets for liver cancer treatments.

## INTRODUCTION

- ATGL is an enzyme in lipolysis that catalyzes the breakdown of triglycerides to fatty acids (1).
- While ATGL can provide an alternative energy source for the cancer cells in the form of fatty acids, too much fatty acid oxidation could risk putting excessive stress onto the cells (2).
- Lipid droplets store triglycerides which can be used for fatty acid synthesis (1).
- Oleate is an unsaturated fatty acid that contributes to lipid droplet formation and lipolysis (3).
- The mechanism behind how ATGL overexpression in cells interacts with chemotherapy agents is not well understood.

## METHODS

- pLJM1 and hATGL lentivirus was created using HEK293T cells and then transduced into Hep3B, HepG2, and HUH7 cells and selected at 0.1 µg/mL puromycin.
- Validation of the lentivirus was done by Western blots, which were loaded with 30 µg of protein, and qRT-PCR.
- Cells were plated at 100,000 cells per well in a 12 well plate and were lipid loaded with 100 µM oleate overnight. The wells were stained with MitoTracker Deep Red and Bodipy 498/503 as mitochondrial and lipid droplet biomarkers and observed with a Nikon A1Rsi Confocal microscope.
- Cell were plated at 5,000 cells per well in a 96 well plate and viability was tested via Alamar Blue after treatment with varying doxorubicin concentrations (0-10 µM) for 24 hours under standard or 100 µM oleate supplemented conditions.
- Data is presented as mean plus or minus standard deviation and analyzed by student's T-test.

## RESULTS

**Figure 1. Visual of the function of ATGL.** Triglycerides (TAGs) are broken down by ATGL into diacylglycerols (DAGs) and a fatty acid.

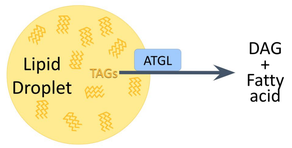
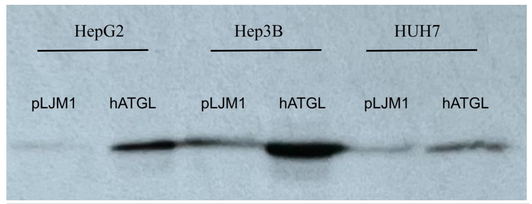



**Figure 2. Validation of lentivirus transduction into HepB3, HepG2, and HUH7 cells**. ATGL overexpression was seen in the cell line as (**A**) a western blot exhibited darker bands with protein containing ATGL over-expression in HepG2, HepB3 100. (**B**) Real time PCR was done to confirm the overexpression of hATGL in the cell lines.

A.

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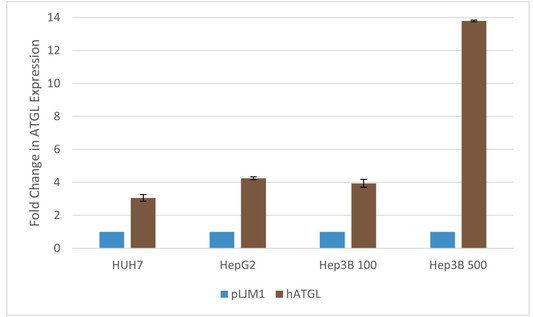
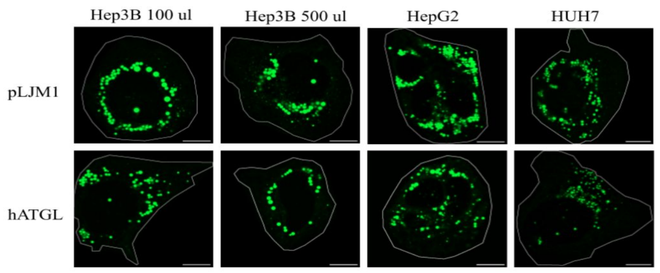



**Figure 3. Imaging of lipid droplets on each cell after eighteen hours of lipid loading.** Plates were stained with Bodipy for thirty minutes prior to imaging. (**A**) An image of each cell type with the Bodipy marker on the lipid droplets. (**B**) Individual cells were examined to quantify the area of lipid droplets per cell.

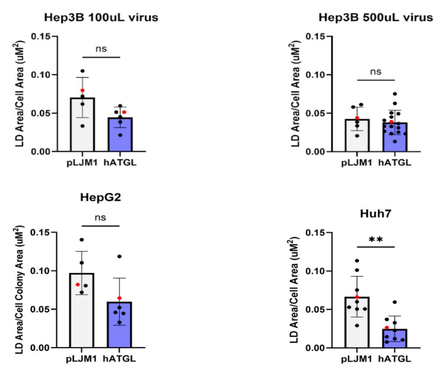
A.




*Scale bar on each image is 10 µm

- **Visually less lipid droplets in hATGL cells compared to control.**

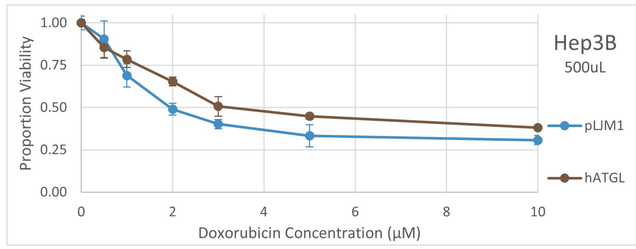
B.




- **Average lipid droplet area per cell is larger in control for all cell lines.**
- **Different amounts of lentivirus added to Hep3B yielded vastly different results.**

**Figure 4. Lethal dosage of 50% for all cell lines under both basal and oleate supplemented conditions.** All cell lines were treated with doxorubicin at various concentrations (0-10 µM), (**A**) Sample graph showing doxorubicin response as proportion viability from vehicle control and (**B**) doxorubicin dosage needed to kill 50% of cells.

A.

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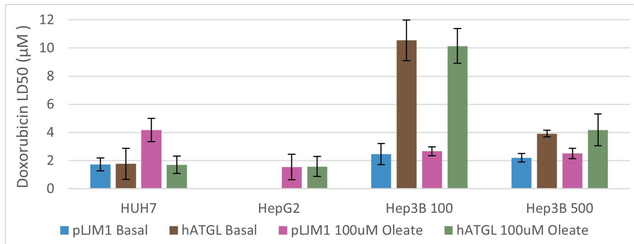


- **HepG2 had a slight increase in LD50 in oleate substituted conditions.**
- **HUH7 hATGL experienced slightly higher LD50 in the basal conditions, but a lower LD50 in oleate conditions.**
- **Overexpression of hATGL at both concentrations (100 and 500 µL) in Hep3B cancer cells decreased doxorubicin effectiveness.**

## CONCLUSIONS

- **Mechanisms that could lead to the desensitization of chemotherapeutic agents observed in this experiment are:**
  - **An increase in p-Akt phosphorylation, which increases liver cancer cell growth, is correlated with ATGL overexpression (2).**
  - **Oleate, as an unsaturated fatty acid, has been shown to decrease apoptosis and cell damage (4). Therefore, ATGL allows cells to utilize oleate to further reduce chemotherapy sensitivity.**
    - **Addition of oleate can reduce the cytotoxicity induced by other fatty acid types, like stearate (5).**
- **Lipid droplet area was higher for pLJM1 cells.**
  - **ATGL breaks down triglycerides, the molecules stored in lipid droplets. Therefore, ATGL overexpression depletes lipid droplet storages, leading to the decreases in surface area seen in Fig 3A.**
- **Overall, ATGL overexpression increases the drug resistance to chemotherapy in Hep3B, HepG2, and HUH7 cancer cells.**
- **In Hep3B 500 µL ATGL cells, there was a distinct upper limit where the ATGL overexpression was limited in presenting further desensitization.**

## FUTURE DIRECTIONS

- **Test the effects of hypoxic conditions on ATGL overexpressed cells.**
- **Alter media conditions (i.e, metabolite levels) to more closely physiological conditions.**
- **Examine other cancer cell lines.**

## REFERENCES

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