Role of Adipose Triglyceride Lipase (ATGL) in Cell Proliferation

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Abstract

Previously viewed simply as a static form of energy storage, the lipid droplet has recently become the focus of intense research due to the discovery of its involvement in numerous cellular metabolic processes. It is currently hypothesized that regulation of lipid droplet metabolism within the cell may have significant effect on cell signaling and disease development, including diabetes and cancer. In this project, we investigate a lipid droplet-associated lipase, adipose triglyceride lipase (ATGL), which catalyzes the first step of fatty acid breakdown by converting triglyceride to diglyceride. This lipase has previously been implicated in regulating cellular energetics and possibly cell growth. In this study, we first investigated various methods for plasmid insertion into proliferating cells and established several stable lines expressing ATGL. We show that ATGL overexpression reduces lipid droplet formation in cells, consistent with its catabolic role in lipid metabolism. We further show that addition of fatty acid to culture media attenuates the growth promoted by increased fetal bovine serum (FBS) and that cell growth is reduced with ATGL overexpression especially when grown in the presence of serum. These findings shed light on the links between lipid metabolism and cell growth and may provide evidence that lipid droplet metabolisms may influence cancer development or progression.

Introduction

- **The link between cancer and altered glycolysis has long been established (1) and has received extensive attention in metabolic research.**
- **Increasing evidence in recent studies also shows altered lipid metabolism in cancer cells (2). However, there is a current lack of research in this area.**
- **Adipose triglyceride lipase (ATGL) is a lipid droplet-associated lipase that catalyzes the first step of fatty acid breakdown by converting triglyceride to diglyceride (3).**
- **Therefore, the purpose of this study was to investigate the effects of ATGL overexpression on lipid droplet formation and cancer cell growth.**

Methods

- **Recombinant plasmids containing enhanced green fluorescent protein (EGFP), constructed as described by Tavian et al. (4), were used to create an empty vector and a vector containing human ATGL.**
- **These plasmids were then transfected into AML12 (mouse hepatocyte) cells using Effectene reagent. Cells were screened for plasmid uptake by fluorescence and through selection by the antibiotic Geneticin at 1.5 mg/ml media. One EGFP control cell line and two ATGL cell lines (ATGL-B and ATGL-D) were chosen for experimentation.**
- **Transfected cells were plated at 50,000 cells per well on a 12-well plate and remained in media containing 10% fetal bovine serum (FBS) for 24 hours. They were then treated for 48 hours with media containing 1% FBS, 10% FBS, or 10% FBS with the addition of the fatty acid oleate at 250 uM.**
- **Cells were stained using Oil Red O to observe lipid droplet accumulation (see figure 1).**
- **Cell growth was inferred based on protein presence, measured with a BCA protein assay kit.**
- **Protein levels were converted to growth ratios defined as the fold induction of protein concentration over 48 h. In figure 2, protein levels are normalized to the 1% FBS media treatment, and in figure 3, normalized to the EGFP control for each media treatment. Data are presented as means and standard errors of the mean and analyzed by Student's *t* test, with $p < 0.05$.**

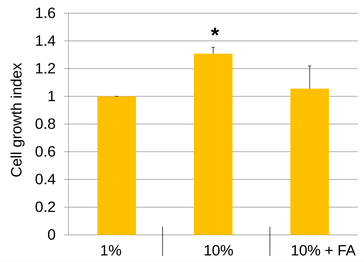


Fig. 2. Cell growth in EGFP control cells. Increasing FBS in media from 1% to 10% caused increased cell growth. Addition of the fatty acid oleate led to a reduction in the effect of increased FBS. Effects were seen in all three cell lines, represented here by the EGFP control.

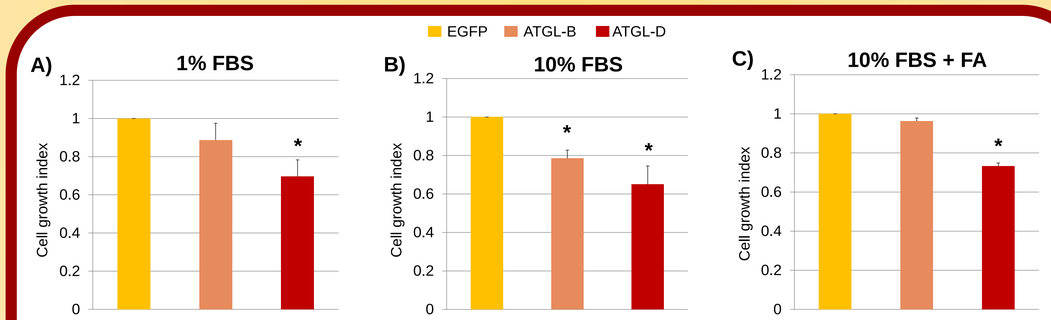
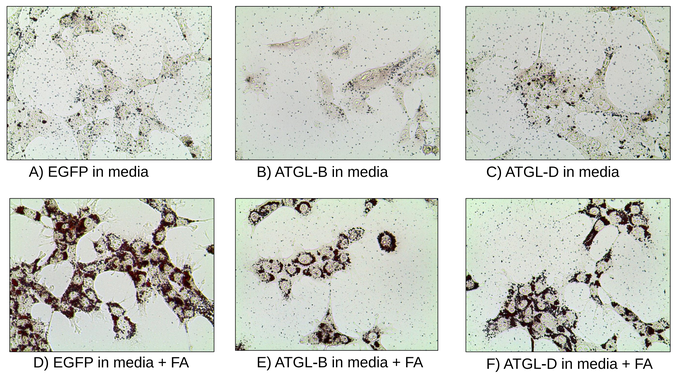


Fig. 3. Cell growth under varying media conditions. ATGL overexpression decreased growth rate relative to the control under each media treatment.

Fig. 1. Oil Red O Staining. (A), (B), and (C) depict cells in 10% FBS media. (D), (E), and (F) depict cells in 10% FBS media with 250 uM oleate.

- A marked increase in size and number of lipid droplets was seen in cells grown with added oleate over those in only 10% FBS media (compare top row to bottom row).
- Decreased number of lipid droplets and intensity of red was seen with overexpression of ATGL (see 1E and 1F), as compared to cells with EGFP control (see 1D).



Conclusion

- **As previously established, ATGL overexpression reduces lipid droplet formation due to its role in cellular triglyceride breakdown.**
- **Higher concentration of FBS in media increased cell growth, but addition of fatty acid attenuated this increase.**
- **ATGL overexpression decreased cell growth relative to the control under conditions of 1% and 10% FBS, as well as 10% FBS with fatty acid.**
- **These findings elucidate the relationship between lipid metabolism and cell growth and contribute to the increasing body of knowledge that supports lipid metabolism as a target for regulation of cancer development and progression.**

Acknowledgements

I would like to thank my mentor, Doug Mashek, as well as Aishwarya Sathyanarayan and Mara Mashek, with whom I worked closely throughout the past year. Without their guidance, support, and patience as I undertook this research, this project could not have been completed. Thank you to Mallory Franklin, whose tireless work yielded the plasmids used in these experiments, and to all the other members of the Mashek lab for welcoming me so warmly. Finally, thanks to Bill Ganzlin, Chi Chen, and the Research Apprenticeship Program for their support.

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