Role of Serum Starvation in Regulating the mRNA Expression of Microtubule-Associated Protein 1 Light Chains

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INTRODUCTION

Autophagy is a homeostatic process that has both tumor-promoting and tumor-suppressing activities. It supports tumor growth by generating nutrients for survival under deprived conditions. In tumor-suppressing autophagy, oncogenic nutrients are degraded in order to diminish tumor growth. Autophagy is induced by serum starvation of cells. Serum is blood plasma that is used in media to support the growth of cells in cell culture.[1]

Microtubule-Associated Protein 1 Light Chains (MAP1 LC3s) are proteins found in the membranes of autophagosomes and are autophagic regulators. LC3A, LC3B, and LC3C are paralogous genes that differ in structure and function. They are derived from the ancestral gene of LC3.[1]

LC3B and LC3C-dependent autophagy are important in the oncogenic process during the development of clear cell renal cell carcinoma (ccRCC), the most common and malignant type of kidney cancer.[1]

ccRCC tumors are characterized by loss of the von Hippel-Lindau (VHL) tumor-suppressor gene. VHL induces expression of LC3C, which exercises tumor-suppressing activity and inhibits the expression of LC3B. LC3B promotes oncogenic autophagy. Therefore, the loss of VHL decreases the expression of LC3C and increases the expression of LC3B, both of which leads to increased oncogenic activity and tumor growth in ccRCC.[1]

The mechanism of LC3C tumor-suppressing autophagy is not well understood. Because it is a human specific protein, LC3C cannot be studied in animal models.[1] In order to gain an understanding of the mechanisms of LC3C tumor-suppressing autophagy, we investigated the biological conditions and mechanisms of how the expression of LC3s is regulated, specifically how induction of mRNA expression is regulated by serum starvation and how that differs between LC3B and LC3C.

We hypothesized that serum starvation induces mRNA expression of LC3s and that there is a difference in mRNA expression between LC3B and LC3C.

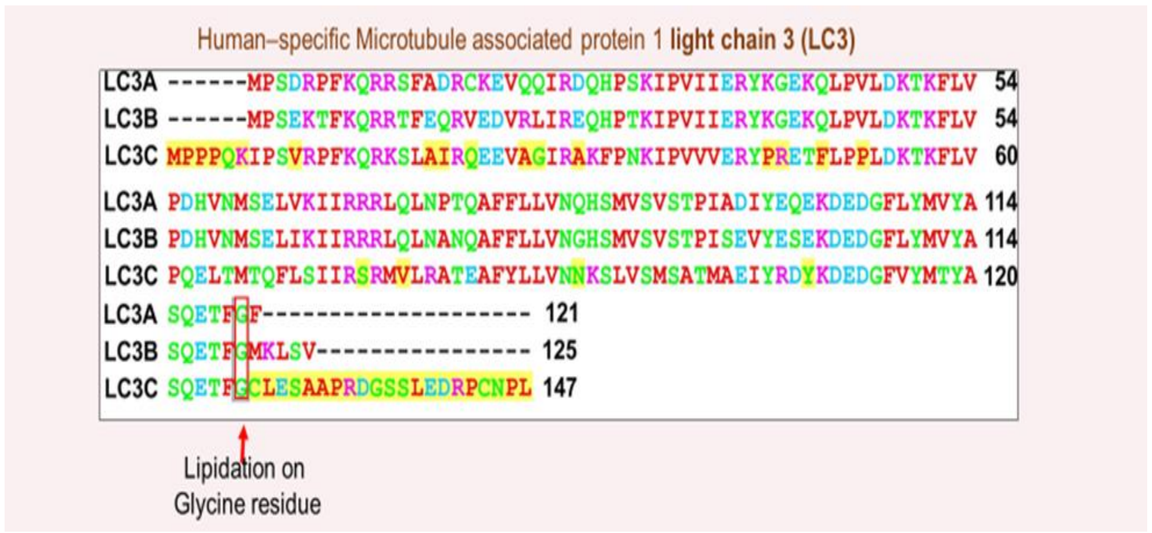

Figure 1: Structure of LC3s.[1]

METHODS

Two different human cell lines, CAKI-1 & 786-0 WT, derived from clear cell renal cell carcinoma tumors that express wild VHL were serum-starved for different lengths of time (6 hours(H), 12H, 24H, 30H, 36H, 48H) in media containing 0.1% fetal bovine serum (FBS). Control cells were grown in 10% FBS.

Initially, all cells were plated in media containing 10% FBS. The media of all cells to be serum starved were changed to 0.1% FBS the following morning. Duplicates of both control cells and serum-starved cells were collected at each time point (6H, 12H, 24H, 30H, 36H, 48H) since the changing of the media.

The mRNA was then extracted from the collected cells, followed by synthesis of cDNA using RT-PCR and the running of qPCR using LC3B and LC3C primers. Finally, the results were analyzed using Excel to determine the fold change of the mRNA levels of LC3B and LC3C expressed by duplicate cells in each serum-starved condition (time) as compared to their respective controls.

A total of six experiments were conducted; 3 biological replicate experiments (n=3) for each cell line. The data from the 3 different experiments of each cell line were combined by averaging the fold change of the mRNA levels of LC3B and LC3C expressed by duplicate cells in each serum-starved condition as compared to their respective controls. The combined data for each cell line was statistically analyzed by running one-way ANOVA tests to determine if there was any significant induction of mRNA expression of LC3B and LC3C in serum starved cells compared to their respective non-serum starved cells and to determine whether there was a difference in induction of mRNA expression between LC3B and LC3C.

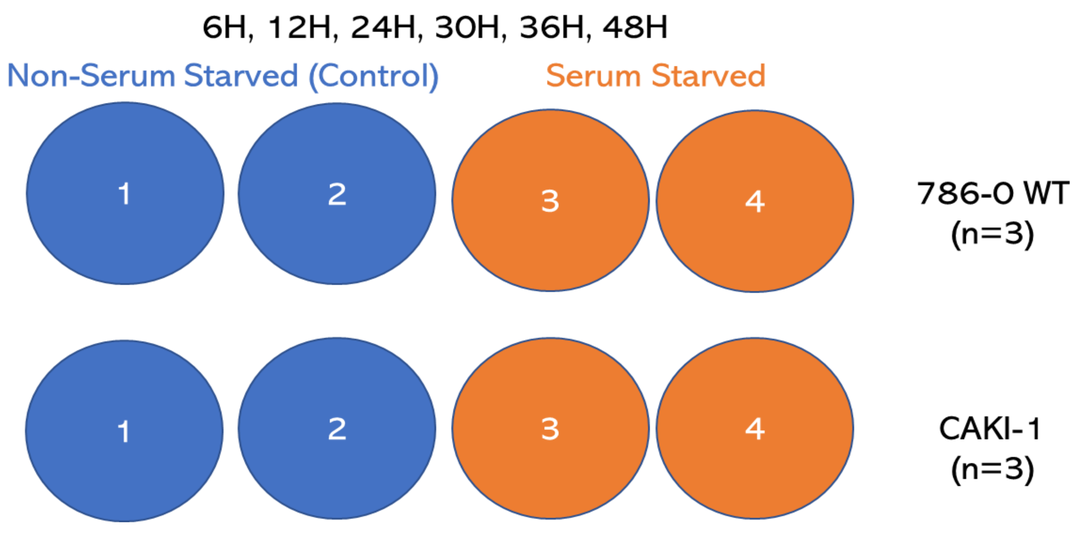

Figure 2: Experimental Setup. For each time point, mRNA was extracted from 4 plates of 786-0 WT cells and 4 plates of CAKI-1 cells. For each cell line, 2 plates contained serum starved cells and 2 plates contained non-serum starved cells.

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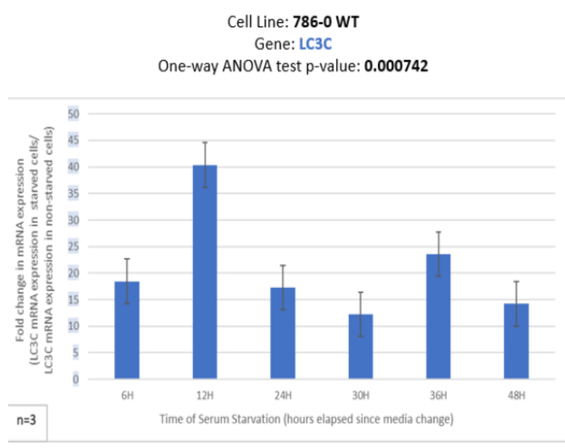
RESULTS



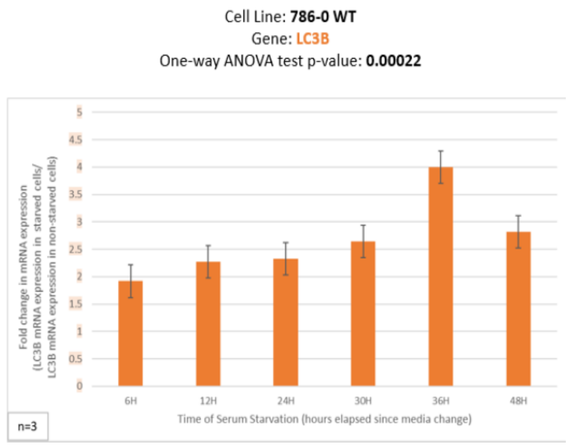
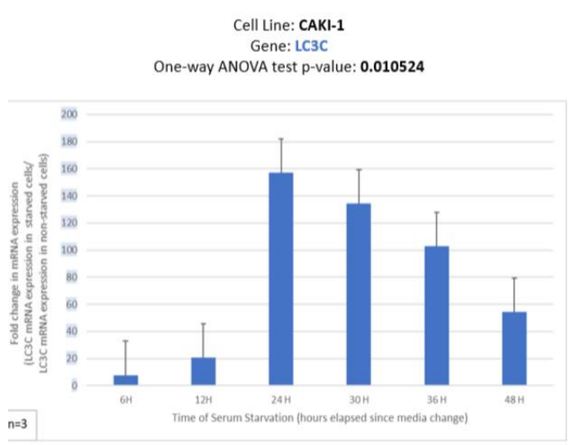


Figure 3: mRNA expression of LC3s in 786-0 WT Serum Starved Cells Normalized to Respective Non-Starved Cells (Controls). Note different scale bars for LC3C and LC3B.



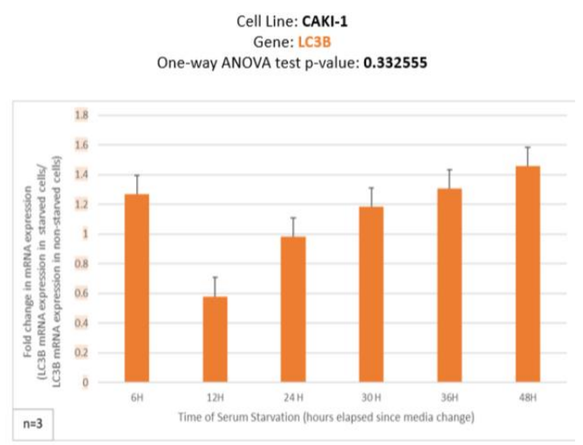
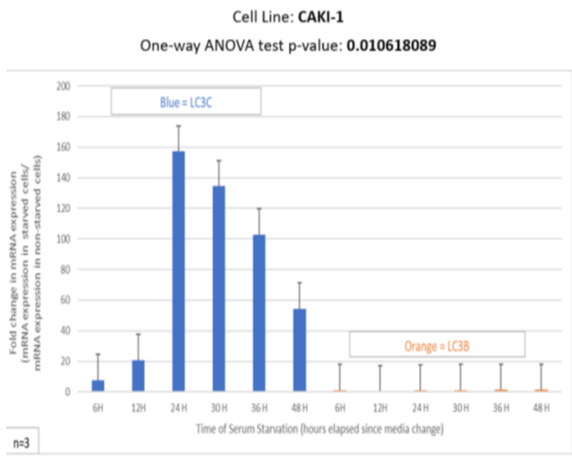


Figure 4: mRNA expression of LC3s in CAKI-1 Serum Starved Cells Normalized to Respective Non-Starved Cells (Controls). Note different scale bars for LC3C and LC3B.



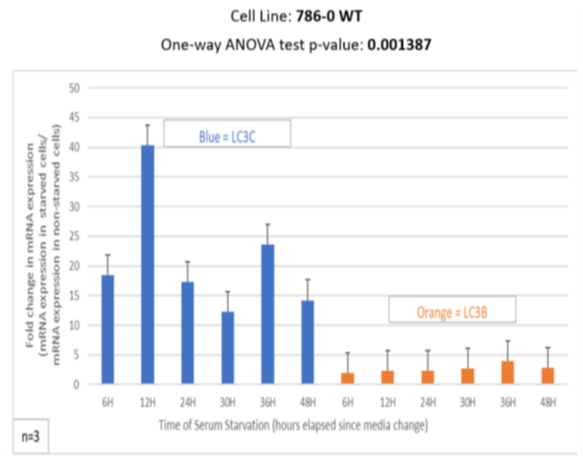


Figure 5: mRNA expression of LC3C & LC3B in Serum Starved Cells Normalized to Respective Non-Starved Cells (Controls). Note scale bars are same.

CONCLUSIONS

Serum starvation of 786-0 WT cells resulted in the significant ($p < 0.05$) induction of mRNA expression of both LC3C ($p = 0.000742$) and LC3B ($p = 0.00022$) compared to non-serum starved (control) cells, as seen in Figure 3. The induction of mRNA expression of LC3C was significantly higher than that of LC3B ($p=0.001387$) in 786-0 WT cells, as seen in Figure 5.

Serum starvation of CAKI-1 cells resulted in the significant ($p < 0.05$) induction of mRNA expression of LC3C ($p = 0.010524$) compared to non-serum starved cells. However, there was no significant induction of mRNA expression of LC3B ($p = 0.332555$), as seen in Figure 4. The induction of mRNA expression of LC3C was significantly higher than that of LC3B ($p= 0.010618089$) in CAKI-1 cells, as seen in Figure 5.

In both 786-0 WT and CAKI-1 cell lines, serum starvation resulted in a long-lasting, stronger induction of mRNA expression of LC3C as compared to LC3B.

SIGNIFICANCE & FUTURE WORK

The strong and long-lasting induction of mRNA expression of LC3C, but not LC3B, demonstrated the induction of LC3C tumor suppressing autophagy by serum starvation. This signifies an important role of the LC3C paralog in regulating autophagy in human cells, which has potential implications for the cellular metabolism of ccRCC tumors.

In terms of the future directions for this study, we are going to determine the effects of serum starvation on the mRNA expression of the genes LC3A, GABARAP, and a variant of LC3C where the most distinguished feature of LC3C, the C-terminal peptide, is removed by introducing a mutation.[1] We are going to test LC3A since it is a part of the LC3 family, and we want to see how the induction of its mRNA expression compares to that of LC3B and LC3C. GABARAP is another potential autophagic regulator that we would like to test since it is similar to LC3s in function.[1] Also, it would be highly fascinating to determine whether the induction of mRNA expression of LC3C becomes like that of LC3B if the peptide sequence of LC3C is altered by excising its additional peptide using CRISPR. Finally, we are going to conduct experiments to determine the effects of serum starvation on the protein expression of LC3A, LC3B, LC3C, LC3C with its additional peptide cleaved, and GABARAP because we want to observe whether the protein expression trends follow the mRNA expression trends upon serum starvation.

REFERENCE

[1]Mikhaylova O, Stratton Y, Hall D, Kellner E, Ehmer B, Drew AF, Gallo CA, Plas DR, Biesiada J, Meller J, Czyzyk-Krzeska MF. (2012). VHL-Regulated MiR-204 Suppresses Tumor Growth through Inhibition of LC3B-Mediated Autophagy in Renal Clear Cell Carcinoma. Cancer Cell, 21 (4). 532-546.

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