Role of glutamine in autophagic lysosome reformation in oral cancer cells

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Abstract

Autophagic lysosomal reformation (ALR), is a mechanism to regenerate functional lysosomes from autolysosomes to maintain lysosome homeostasis. Glutamine acts as an intracellular carbon and nitrogen source for cellular homeostasis, and its uptake, as well as metabolism, is essential for cancer cell survival. In this study, we have found that glutamine starvation (GS) led to an accumulation of a significantly higher number of LC3 puncta suggesting an increase in the formation of autophagosomes. Further, we performed the co-localization analysis using LC3 and LAMP1 (Lysosome-associated membrane protein 1). We found significantly higher colocalization (%) of LC3-LAMP1 in glutamine-starved Cal33 cells compared to control conditions, indicating that glutamine starvation promotes autophagosome-lysosome fusion in oral cancer cells. Next, we transiently transfected mcherry-LAMP1 in Cal33 cells to examine the role of glutamine starvation in ALR. Interestingly, we found that glutamine starvation (10hrs) led to the formation of a higher number of proto-lysosome, as indicated by a more extended tubular structure than the control condition. Pharmacological inhibition of mTOR (rapamycin) and genetic inhibition of clathrin (sh-*CLTH*) and Rab7 (si-*RAB7*) significantly abolished the formation of proto-lysosome in glutamine starved Cal33 cells. However, treatment with dynasore (dynamin 2 inhibitor) leads to the elongation of tubular structures, indicating complete inhibition of proto-lysosome scissions. Conclusively, glutamine is essential for regulating lysosomal homeostasis through ALR in oral cancer.

Objectives

1. To investigate the effect of glutamine starvation in autophagy induction and autolysosome formation in oral cancer cells.
2. To examine the role of glutamine starvation in autophagic lysosome reformation.

Methodology

Cell culture: Cal33 cells were cultured in Dulbecco's minimum essential medium (DMEM) supplemented with 10% fetal bovine serum and 1X penicillin/streptomycin under 5% CO2; 37ºC.

Live Cell Imaging: Post treatment, the transfected cells were visualized under a confocal microscope, the number of LAMP1 positive structure with and without tubulation were observed and were quantified using ImageJ software. Several raw pictures directly captured by the microscope were measured for statistical data acquisition.

Immunofluorescence: Following treatment Cal33 cells were fixed, permeabilized and treated with primary and secondary antibodies to visualize the desired proteins.

Result

1. GS promotes autolysosome formation in oral cancer cells

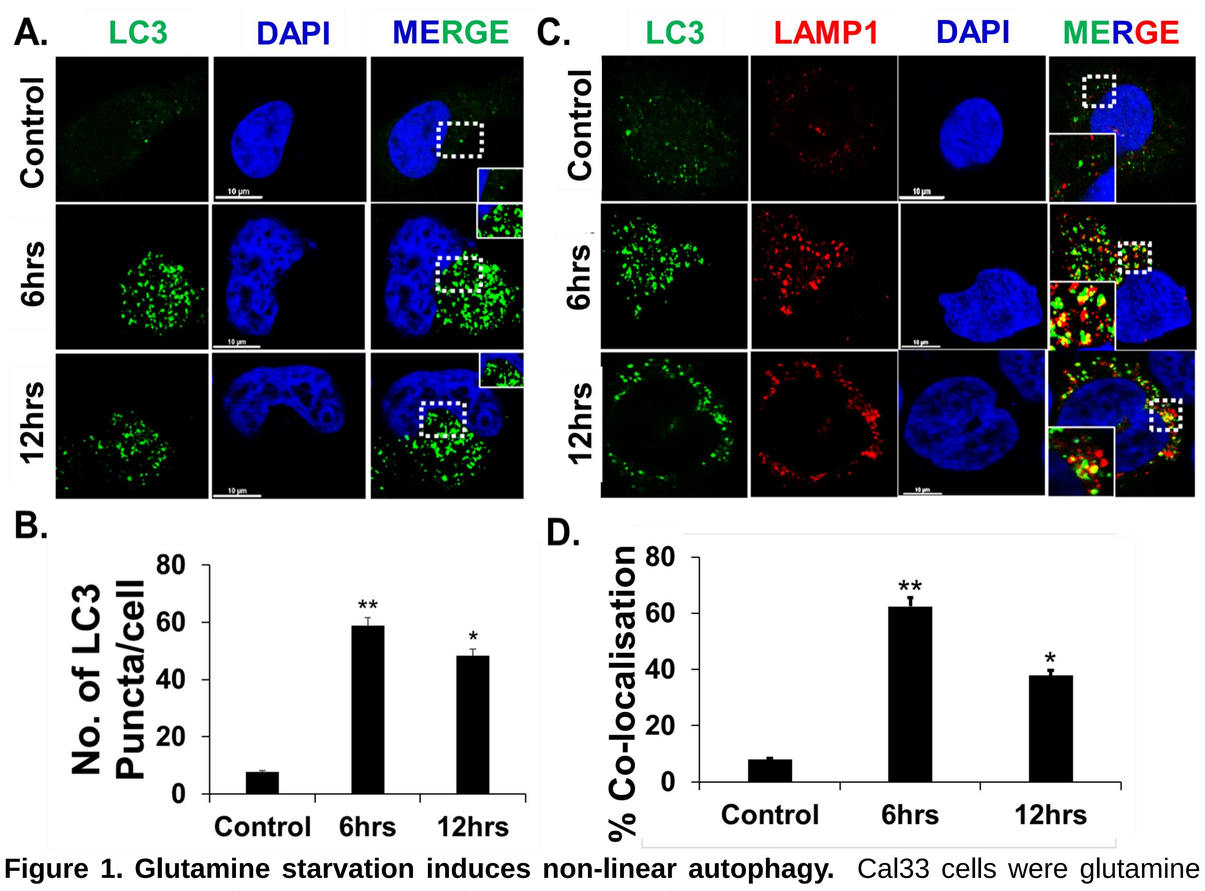

Figure 1. Glutamine starvation induces non-linear autophagy. Cal33 cells were glutamine starved for 24 h followed by immunofluorescence analysis using LC3, and LAMP1 (A-D). DAPI is used to stain nucleus. The data were reported as the mean ± S.D. of three independent sets of the experiment and compared to PBS control. *P-value < 0.05; **P-value < 0.01 were considered significant.

2. GS enhances lysosomal activity in oral cancer cells

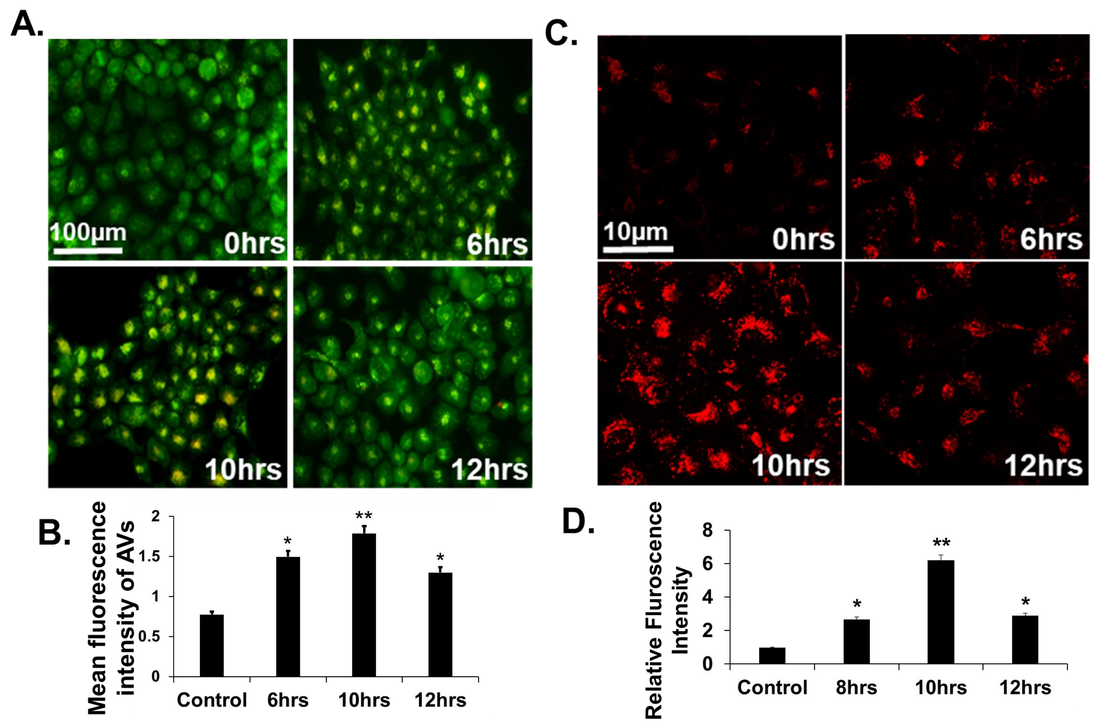

Figure 2. Fluctuation of lysosomal activity during glutamine starvation. Cal33 cells were glutamine starved for 6hrs, 10hrs & 12hrs followed by immunofluorescence analysis using acridine orange and lysotracker (A-D). The data were reported as the mean ± S.D. of three independent sets of the experiment and compared to PBS control. *P-value < 0.05; **P-value < 0.01 were considered significant.

3. GS triggers lysosomal tubulation in oral cancer cells

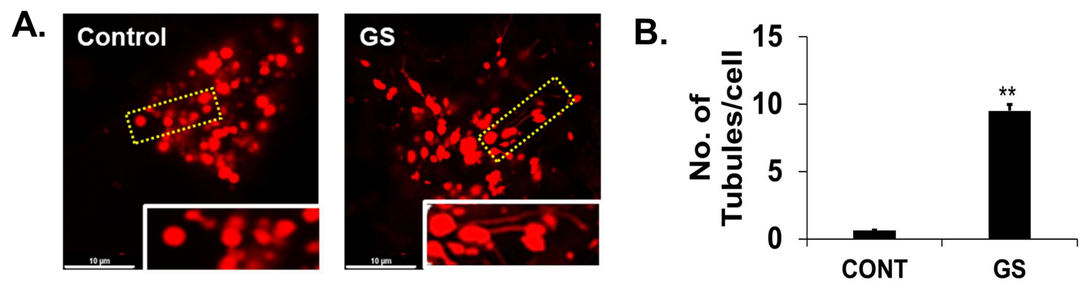

Figure 3. Glutamine starvation induces tubulation. Cal33 cells transiently transfected with mcherry-LAMP1 were glutamine starved for 10 h followed by live cell imaging using confocal microscopy (A-B). The data were reported as the mean ± S.D. of three independent sets of the experiment and compared to PBS control. **P-value < 0.01; were considered significant.

4. Pharmacological and genetic inhibition of key mediators of ALR attenuates GS induced lysosomal tubulation

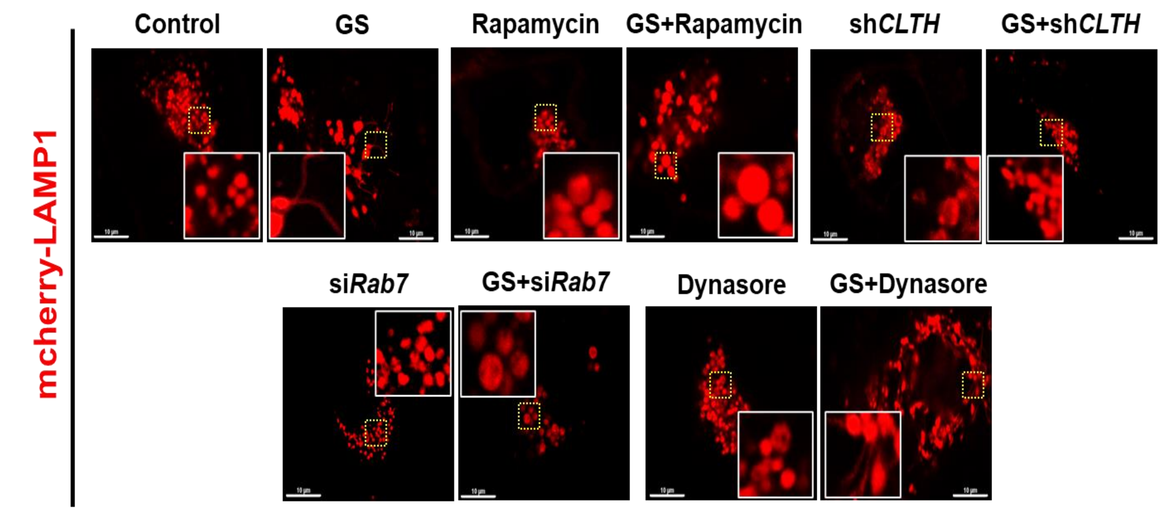

Figure 4. Inhibition of key ALR mediators attenuates lysosomal tubulation. Representative confocal image of Cal33 cells transfected with mcherry-LAMP1 followed by pharmacological inhibition [using (Rapamycin; 150 nM; 10 h), (Dynasore; 40 µM; 10 h)] and genetic inhibition using sh*CLTH* and si*Rab7.* The data were from three independent set of experiments.

Conclusion

The present study unravels the important function of glutamine deficiency in autophagy and lysosomal homeostasis through ALR in oral cancer cells. Moreover, further study on the regulatory mechanism involved in autophagy and ALR could be helpful in treating oral cancer cells.

References

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