The Research in Human Genetic Diseases

Student(s)
GONCA ŞENEROĞLU

Faculty Member(s)
ASSOC. PROF. ÖZLEM KUTLU

PURE
PROGRAM FOR UNDERGRADUATE RESEARCH

ABSTRACT

We have investigated Nonalcoholic steatohepatitis (NASH) and GAUCHER DISEASE human genetic diseases that are thought to be associated with autophagy. This work was supported by The Scientific and Technological Research Council of Turkey (TUBITAK)-3501 National Young Researcher Career Development Program (No: 112T130) and Sabanci University Internal Research Grant (No: I.A.SN-17-01698).

NASH (Nonalcoholic Steatohepatitis)

Gaucher Disease

AUTOPHAGY

- Autophagy is a tightly regulated pathway with an important housekeeping role, allowing cells to eliminate damaged or harmful components through catabolism and to recycle them to maintain nutrient and energy homeostasis. Autophagy is also a major protective mechanism which allows cell survival in response to multiple stress conditions such as nutrient or growth factor deprivation, hypoxia, reactive oxygen species (ROS), DNA damage or intracellular pathogens.

NASH

- NASH is one of the fatty liver types that occur when the liver accumulates fat (steatosis) for reasons other than excessive alcohol use.
- NASH is a serious form of fatty liver disease. As NASH patients become fattened in the liver cells, liver cells swell and inflammation increase.
- Researchers are not sure why some people with NAFLD have NASH and others have simple fatty liver. Research suggests that certain genes may play a role[1].
- People with NAFLD are more likely to have NASH if they have one or more of the following conditions:
  -obesity, especially with a large waist size
  -type 2 diabete

GAUCHER DISEASE

- Lysosomal Storage Disease
- The disease is caused by a recessive mutation in the acid-β glucocerebrosidase (GCase) gene. As a result, defective hydrolysis of glucosphingolipids results in the accumulation of glucosylceramide (GC) and glucosylsphingosine (GS) in liver, spleen, macrophages.

OBJECTIVES

- Our goal for NASH disease is to monitor the effect of autophagy during the formation phase. Investigate possible targets for drug treatment in this disease.
- To reduce the increased pH value due to the fault occurring in autophagic pathways in target Gaucher patient cells by biocompatible nanoparticle treatment.

MATERIAL-METHODS

NASH	GAUCHER
The tissues from the liver of NASH patients were homogenized	Primary Cell Culture
RNA isolation	Cell Passaging
RT(Reverse Transcription) PCR-cDNA Synthesis	Cell Seeding
qPCR – Gene Expression	Nanoparticle Treatment
ΔΔCT- Data Analysis	Lysosomal pH

PROJECT DETAILS

NASH

Ten NASH patients and five healthy control who underwent liver biopsy at Cukurova University Medical Faculty were evaluated prospectively. Liver tissue samples from NASH patients were homogenized and total RNA was isolated. cDNA was synthesized and analyzed for expression of Beclin1, ATG5, ATG7 by using Quantitative RT-PCR method.

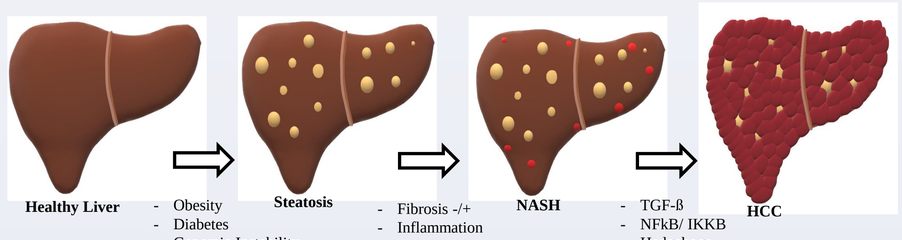

Figure 1. Further stages of NAFLD [2]

GAUCHER DISEASE

Gaucher cells show an increase in lysosomal pH due to increased lipid content. Our goal is to reduce the increasing pH value in gaucher cells by using nanoparticles. The samples from patient tissues were taken from Hacettepe University Medical Faculty.

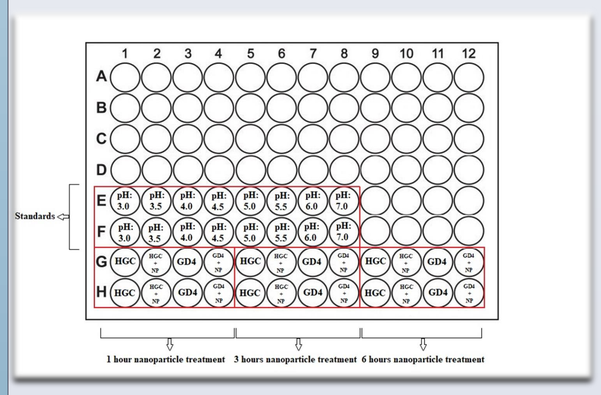

Figure 2. Lysosomal pH Experiment Well Plan

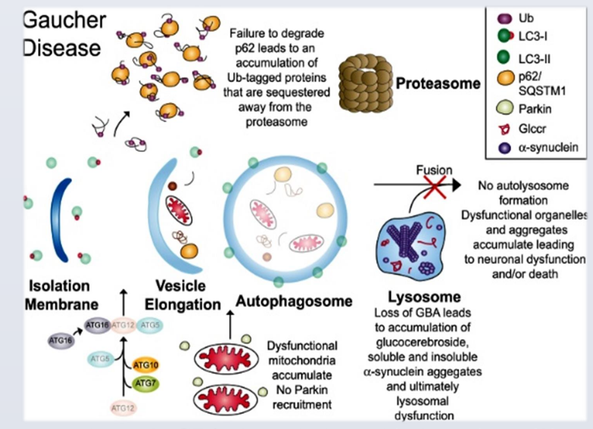

Figure 3. GD's Association with Autophagy[3].

With HGC (Human gingivial cell) cells pH curve buffer (prepared with MES hydrate) was performed with 3-3.5-4-4.5-5-5.5-6 and 7. Monencin and nigericin was freshly added to aliquoted pH buffers. Lysosensor was used to measure the Ph values of cells. Cells were washed with PBS. pH curves the solutions with indicated pH values were given to wells and for patient cells and control HGC cells with and without overexpression as pH assay control measurement buffer was given.

CONCLUSIONS

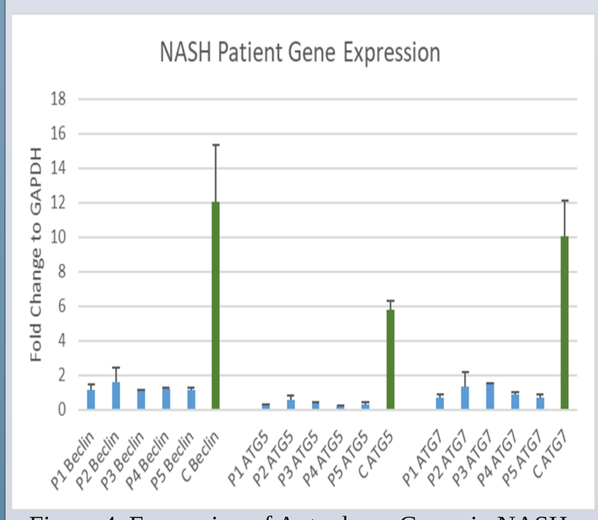

Figure 4. Expression of Autophagy Genes in NASH and Comparison with Healty Cells

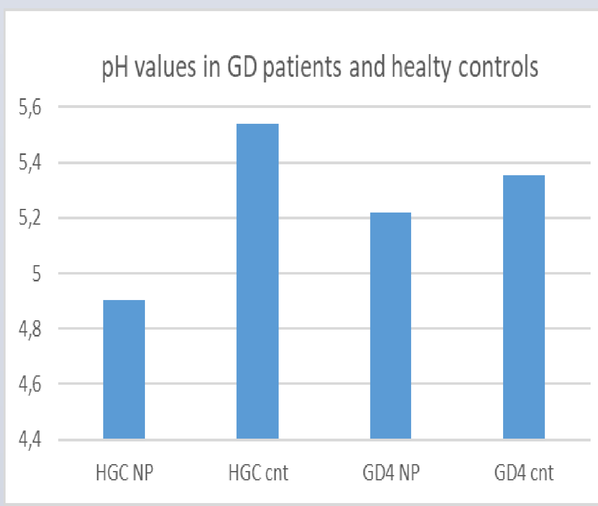

Figure 5. Lysosomal pH Experiment

REFERENCES

[1] Dixon, J. B., Bhathal, P. S., & O'brien, P. E. (2001). Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology, 121(1), 91-100.

[2] Ozlem Kutlu, Humeyra Nur Kaleli, Ebru Ozer (2018). Molecular Pathogenesis of Nonalcoholic Steatohepatitis (NASH)-related Hepatocellular Carcinoma. *Can. J. Gastro and Hepatology.*

[3] Zaffagnini, G., & Martens, S. (2016). Mechanisms of selective autophagy. *Journal of molecular biology*, *428*(9), 1714-1724.