Glycogen storage disease type III as a model to study glycogen driving tumor development

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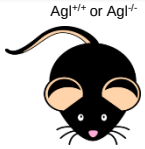
INTRODUCTION

Glycogen storage disease type III (GSDIII) is a disorder caused by the lack of the glycogen debranching enzyme (GDE) protein (encodes by *Agl*), which leads to prominent abnormal structured glycogen accumulation in hepatocytes (called limit dextrin) and hypoglycemia. Hepatic fibrosis and hepatocellular carcinoma (HCC) have been also observed in about 5% of GSDIII patients in the early adulthood. We hypothesized that the hepatic glycogen accumulation might be promoting tumor growth in these patients

OBJECTIVE

- **Study of the development of hepatic tumors in a mouse model of GSDIII**
- **Characterization of glucose and lipid metabolism in the liver of GSDIII mice**
- **Impact of glycogen accumulation on the cellular stress in GSDIII liver**

MATERIALS AND METHODS



14 month-old

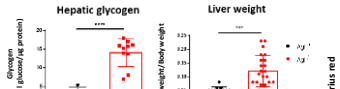
Histology (Sirius red, ORO, TEM)

Metabolic analysis (glycogen, G6P, lactate, AFP, triglycerides (TG), cholesterol)

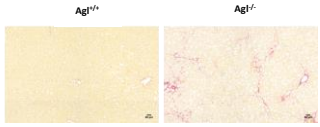
Molecular analysis

RESULTS

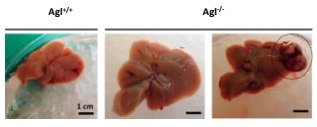
Glycogen accumulation, hepatomegaly, fibrosis and HCC in Agl$^{-/-}$ mice

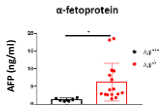


- Increased hepatic glycogen and liver weight in Agl$^{-/-}$ mice



- Fibrosis (stained in pink) in Agl$^{-/-}$ livers

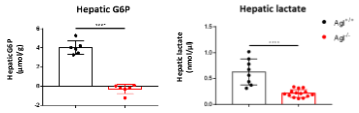




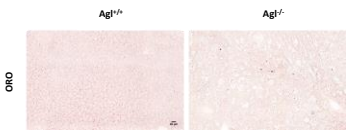
- 30% of Agl$^{-/-}$ mice developed HCC, in accordance with increased α-fetoprotein (AFP) levels in Agl$^{-/-}$ mice

T-test statistical analysis * p<0.05; *** p<0.001; **** p<0.0001

Reduced glycolysis and lipid content in Agl$^{-/-}$ livers



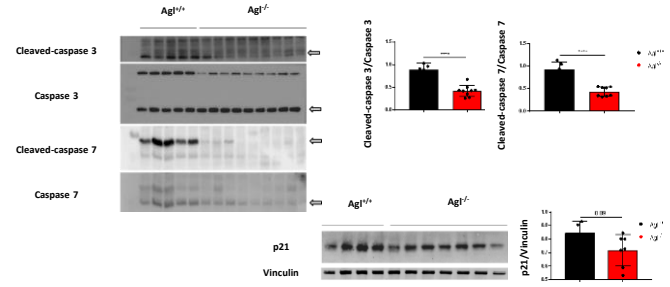
- Reduced hepatic glucose-6-phosphate (G6P) and lactate levels in Agl$^{-/-}$ mice
- Absence of Warburg effect in Agl$^{-/-}$ livers



- Oil red O (ORO) staining show less lipid content (red spots) in Agl$^{-/-}$ mice, in accordance with a decrease in the *de novo* lipogenesis gene expression (not shown)

T-test statistical analysis *** p<0.001; **** p<0.0001

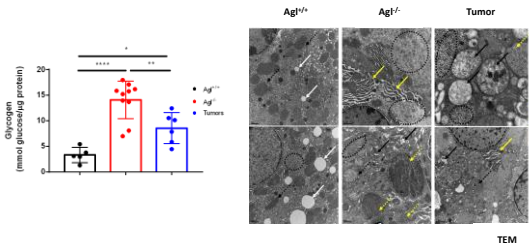
Reduced apoptosis and increased proliferation in Agl$^{-/-}$ livers



- Decreased cleaved-caspase 3 and 7 indicate reduced apoptosis in Agl$^{-/-}$ livers. Reduced p21 protein levels in these livers suggest an increase in proliferation

T-test statistical analysis **** p<0.0001

The autophagic degradation of glycogen drives tumor formation



- Reduced glycogen content in hepatic tumors of Agl$^{-/-}$ mice
- Representative images of transmission electron microscopy (TEM) show dilated ER in Agl$^{-/-}$ mice and autophagic features in tumors of Agl$^{-/-}$ mice that indicate increased autophagy

T-test statistical analysis * p<0.05; ** p<0.01; **** p<0.0001

CONCLUSION

We have observed tumor formation with fibrosis without lipid accumulation in the livers of Agl$^{-/-}$ mice. Moreover, there is not Warburg effect in these mice and therefore glycogen might be the fuel used by hepatocytes to proliferate. Glycogen accumulation is also promoting ER dilatation. ***These results indicate the importance of glycogen as energetic substrate for tumor generation and the relevance of GSDIII mouse model to study new mechanisms behind HCC formation***

TAKE HOME MESSAGE

Our data support a clear role of glycogen fuelling tumor formation through autophagy activation