Role of N-acetylaspartate in hepatocellular carcinoma

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ABSTRACT

N-acetylaspartate (NAA) is a mitochondrial metabolite synthesized through the catalysis of the enzyme L-aspartate N-acetyltransferase (NAT8L), predominantly expressed in the brain. Once produced it can be exported in the cytosol where it is metabolized by the aspartoacylase (ASPA) enzyme in aspartate and acetate or it can be extruded from the cell, becoming an important signaling molecule for recipient cells. Alterations in NAA pathway are associated with several disorders of the central nervous system, but recent evidence has also demonstrated the involvement of NAA metabolism in pathological conditions outside the brain, such as in diabetes, obesity and cancer (Daniele et al., 2020; Moffett et al., 2007). In particular, NAT8L expression is increased in lung and ovarian cancer and negatively correlated with patients survival (Bogner-Strauss, 2017). Considering that the majority of lipid, carbohydrate, and amino acid metabolic pathways occur in the liver, which has an essential role in systemic metabolism, we have verified whether alteration of NAA synthesis impacts the metabolic reprogramming of hepatocellular carcinoma (HCC).

AIM: Has NAA a role in the metabolism of hepatocellular carcinoma?

NAT8L silencing promotes proliferation and migratory capability in HepG2 cell line

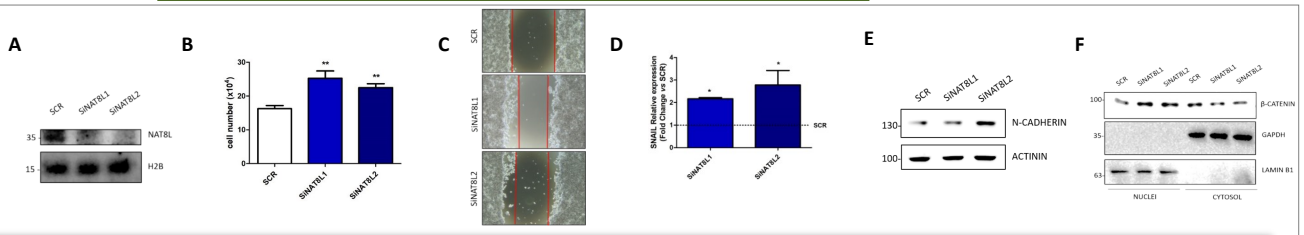

FIGURE 1: NAT8L downregulation (**A**) is associated with increased proliferation (**B**) and migratory capability in HepG2 cell line, as demonstrated by wound healing assay (**C**) . Epithelial-to-mesenchymal transition markers are also affected: SNAIL mRNA expression level is increased (**D**), N-cadherin protein level is increased (**E**) and β-catenin is more present at the nuclear level (**F**)

NAT8L silencing increases glucose demand in HepG2 cell line

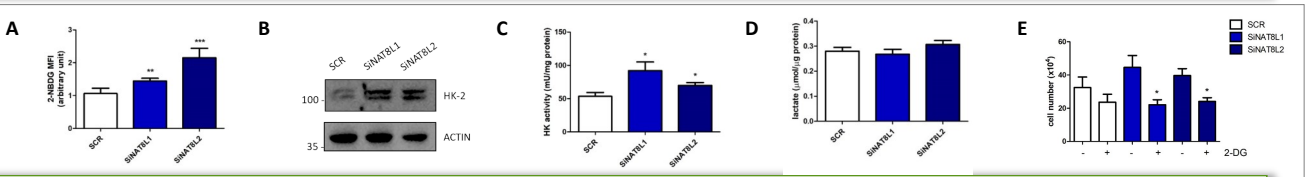

FIGURE 2: NAT8L-silenced cells show an increase in glucose uptake (**A**) and glycolytic rate, as demonstrated by increased levels of protein (**B**) and activity (**C**) of hexokinase (HK). However, extracellular lactate levels are not increased (**D**). Moreover, by inhibiting glycolysis with 2-deoxyglucose (2-DG) HepG2 silenced for NAT8L lost their proliferative advantage demonstrating that they required glucose for efficient proliferation (**E**).

Glucose is efficiently channelled into the pentose phosphate pathway in NAT8L-silenced cells

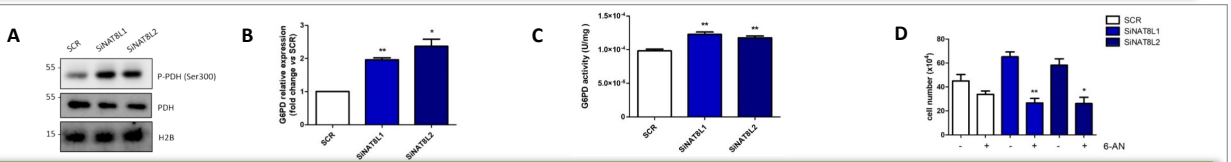

FIGURE 3: The inhibitory phosphorylation of Pyruvate Dehydrogenase (PDH) (**A**) demonstrated that pyruvate is not imported into the mitochondria in NAT8L-silenced cells. The increased levels of glucose-6-phosphate dehydrogenase (G6PD) expression (**B**) and activity (**C**) indicate that glucose is channelled into the pentose phosphate pathway. This hypotesis is supported by the data obtained with the inhibitor of pentose phosphate pathway 6-aminonicotinamide (6-AN), which significantly affected the proliferation of NAT8L-silenced cells with no effect on control ones (**D**) .

NAT8L silencing results in decreased mitochondrial content in HepG2 cell line

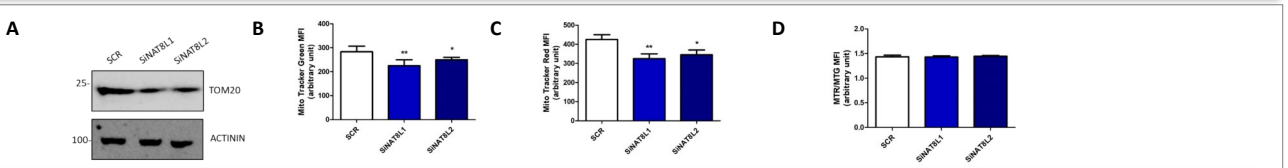

FIGURE 4: Mitochondrial content is decreased in NAT8L-silenced cells, as demonstrated by Western Blotting analysis of TOM20 levels (**A**) and FACS analysis throught MitoTracker Green probe (**B**). However the ratio between MitoTracker Green (**B**) and Red (**C**) demonstrated that mitochondria of NAT8L-silenced cells are functional as those of wildtype cells (**D**).

Mitochondrial metabolism also supports the proliferative advantage of NAT8L-silenced cells

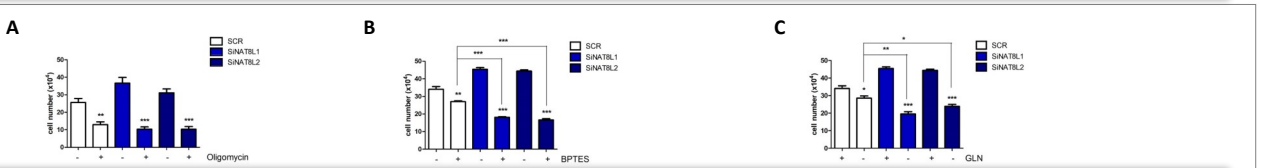

FIGURE 5: NAT8L-silenced cells lost their proliferative advantage upon oligomycin treatment (**A**). Glutamine is fundamental for the increased proliferation of NAT8L-silenced cells, as demonstrated by the treatment with BPTES, a glutaminase inhibitor, (**B**) or the incubation in a "glutamine free medium" (**C**).

NAT8L is downregulated in HCC *in vivo* and the increment of NAA levels, obtained by NAT8L overexpression or NAA exogenous administration, decreases proliferation rate in HepG2 cells

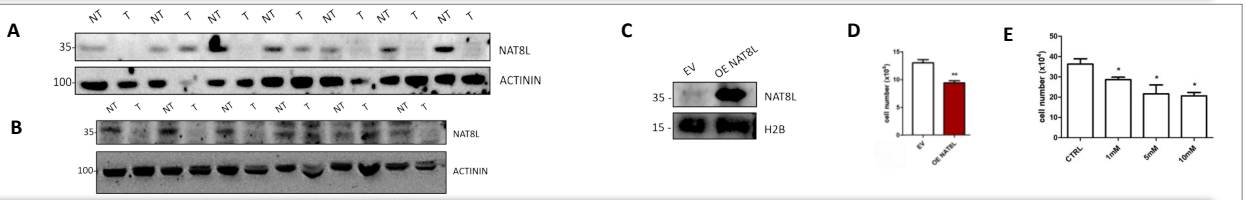

FIGURE 6: Western Blotting analysis demonstrated that NAT8L is downregulated in biopsies from HCC patients (**A**) and a mouse model of HCC (**B**). The overexpression of NAT8L in HepG2 cells (**C**) reduced the cell proliferation rate (**D**). Exogenous NAA treatment decreased the proliferation rate in a dose-dependent manner (**E**).

CONCLUSION

Our results suggest that NAA plays an important role in the metabolic reprogramming of hepatocellular carcinoma, as downregulation of NAT8L was observed in HCC tumor samples. In particular, the downregulation of NAT8L in HepG2 cells resulted in increased proliferation and boosted the pentose phosphate pathway flux, whereas its overexpression inhibits the proliferation rate. Future investigations will be addressed to identify metabolic vulnerabilities that can be exposed by NAA pathway deregulation in cancer and the sensitivity to drugs already in use against liver tumors.

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