Role of N-acetylasparrtate in hepatocellular carcinoma

Pamela De Falco, 2Fabio Ciccarone, 3Enrico Desideri, 3Serena Castelli and 4Maria Rosa Cirio

1 Department of Biology, University of Rome "Tor Vergata", Rome, Italy
2 IRCCS San Raffaele Pisana, Department of Human Sciences and Promotion of the Quality of Life, San Raffaele Roma Open University, Rome, Italy

ABSTRACT

N-acetylasparrtate (NAA) is a mitochondrial metabolite synthesized through the catalysis of the enzyme L-asparagine N-acetyltransferase (NATBL), predominantly expressed in the brain. Once produced, it can be exported to the cytosol where it is metabolized by the aspartate-asparagine (ASPA) enzyme in asparate and acylate 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, NATBL expression is increased in lung and ovarian cancer and negatively correlated with patients survival (Bogner-Straus, 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?

FIGURE 1: NATBL 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).

FIGURE 2: NATBL 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 NATBL lost their proliferative advantage demonstrating that they required glucose for efficient proliferation (E).

FIGURE 3: The inhibitory phosphorylation of Pyruvate Dehydrogenase (PDH) (A) demonstrated that pyruvate is not imported into the mitochondria in NATBL 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 hypothesis is supported by the data obtained with the inhibitor of pentose phosphate pathway 6-amino nicotinamide (6-AN), which significantly affected the proliferation of NATBL silenced cells with no effect on control ones (D).

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

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

REFERENCES


CONCLUSION

Our results suggest that NAA plays an important role in the metabolic reprogramming of hepatocellular carcinoma, as downregulation of NATBL was observed in HCC tumor samples. In particular, the downregulation of NATBL 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.