Fatty acids accumulation in intrahepatic cholangiocarcinoma-derived tissue and sera

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Introduction

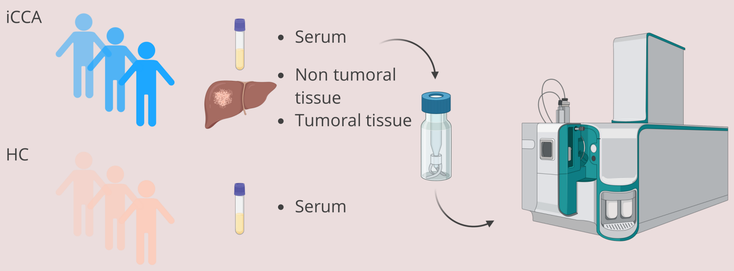
Intrahepatic cholangiocarcinoma (**iCCA**) is the second most common primary hepatic rare malignacy, whose incidence and mortality rate have increased globally over the last 20 years.
Due to a lack of early symptoms and reliable diagnostic markers, patients frequently present at an advanced stage which results in very limited treatment options.

Lipids are crucial for cell survival, being components of biological membranes, energy suppliers via fatty acid oxidation and bioactive mediators.
Notably, altered lipid homeostasis contributes to the development and to the rapid progression of different cancers, and inhibition of lipid biosynthesis has been targeted to reduce tumor growth.
Among primary liver cancers, several studies have described lipidomic deregulation in hepatocellular carcinoma, the most common and studied hepatic form of cancer, while **information on the iCCA lipidomic profile is still lacking**.

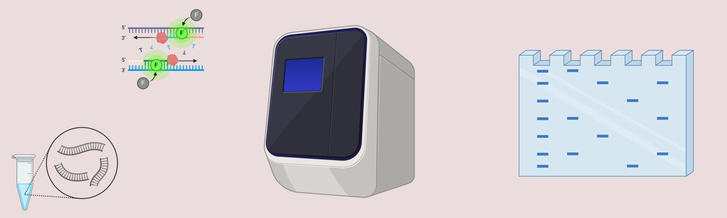
Fatty acid (**FA**) synthesis is misregulated in iCCA while active uptake by specific transporters seems to be enhanced, even though conflicting data exist on the mechanism of FA accumulation.

Methods

In this study, we investigated iCCA lipidome profile by **liquid chromatography-tandem mass spectrometry** (**LC–MS/MS**) analysis of **iCCA tissue** and **matched non-tumor surrounding tissues** from biopsies obtained from patients who underwent surgical resection of iCCA.
Sera from iCCA patients and healthy subjects were also analyzed.



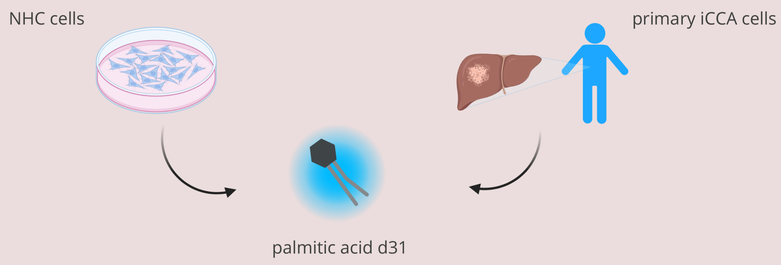
Moreover, several **markers** of the predominant metabolic pathways related to fatty acid metabolism were evaluated by **qPCR** and **Western Blotting**.



qPCR for:
- ACACA
- FABP5
- FABP6
- CD36
- ACADM
- SLC25A20
- SPHK1
- SGPP1
- SGPL1
- ABCC1
- SPNS2
- S1PR3

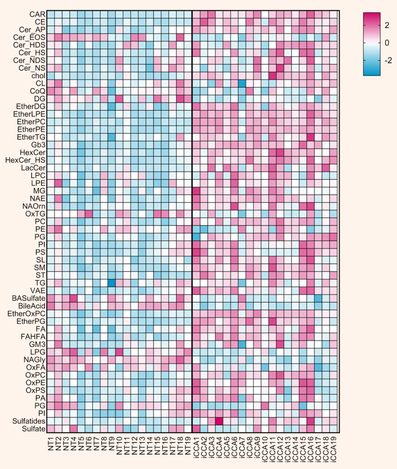
Western Blot for:
- ACADM

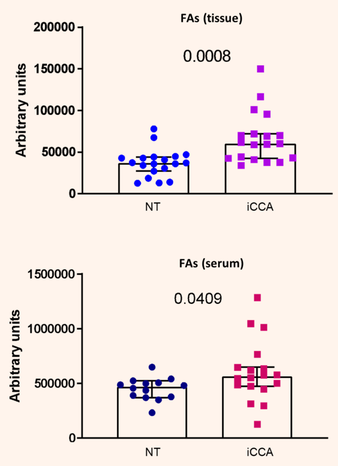
Then, results obtained on iCCA-derived FAs destiny were confirmed through a **quantitative metabolic flux** investigation on **NHC cell cultures** and **primary iCCA cell cultures**.



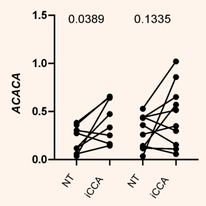
Results

The untargeted lipidomic profile by high-resolution mass spectrometry demonstrated that there was a clear-cut separation between iCCA and NT tissues, as well as between iCCA-derived sera and HC.
In particular, **FAs** predominantly **were enriched** both **in iCCA tissue and serum** compared with NT controls.



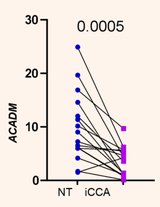


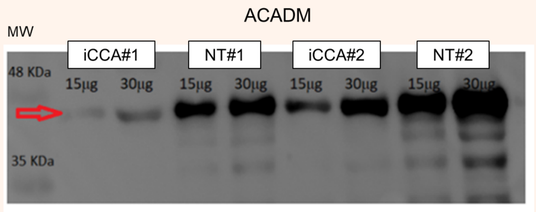
Expression levels of Acetyl-CoA Carboxylase Alpha (**ACACA**), the the rate-limiting enzyme of de novo lipogenesis, were **higher in iCCA tissue** than in NT tissue.



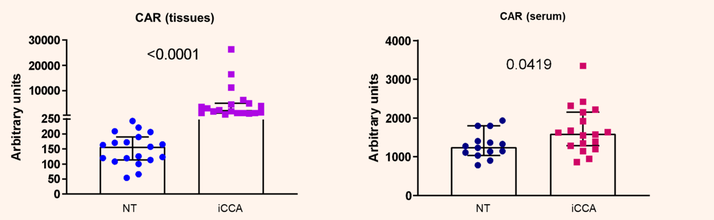
On the other hand, expression levels of several membrane-associated transporters involved in the internalization of FAs, such as Fatty Acid Translocase/Cluster of Differentiation 36 (FAT/CD36), Fatty Acid Binding Proteins (FABPs, in particular FABP5 and FABP6), and Fatty Acid Transport Proteins (FATPs), didn't show an overall significant change.

ACADM (acyl-CoA dehydrogenase medium chain), which catalyzes the initial step of β-oxidation, was about twenty five times **less expressed in iCCA** compared with NT tissue.
Accordingly, its transcription was lower in iCCA tissue than in NT tissue.

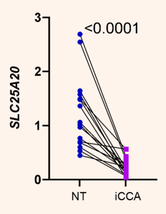




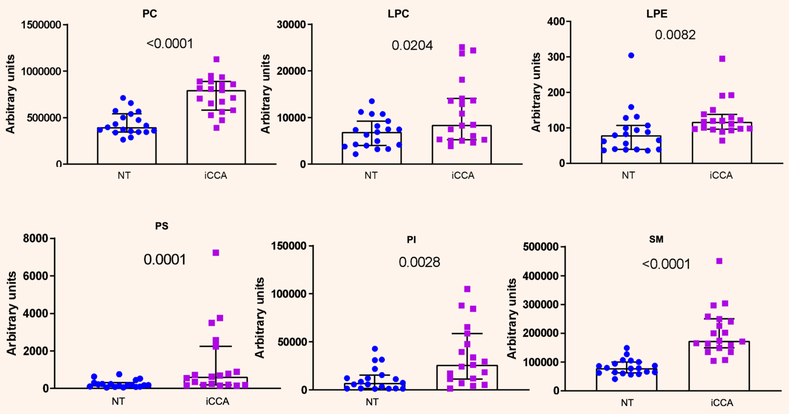
Lipidomic analysis showed that **in iCCA** there was a **large accumulation of acylcarnitine** (**CAR**), which participates in FA translocation to the mitochondria.
This excess of CAR was mirrored in iCCA patients' sera.



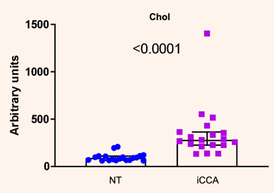
Of note, transcription of the solute carrier family 25 member 20 (SLC25A20), also known as carnitine/acylcarnitine transporter (CACT), a mitochondrial-membrane–carrier protein involved in the transport of CAR into mitochondrial matrix for oxidation, was strongly impaired in iCCA tissue.



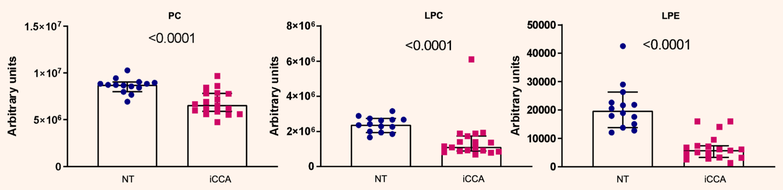
Phospholipids (PL) and sphingolipids (SPL) are the **main components of plasma membranes** that directly derive from FAs.
We observed that several species belonging to these lipid classes were **up-regulated in iCCA** compared with NT tissue, such as phosphatidylcholine (PC), lysophosphatidylcholine (LPC), lysophosphatidylethanolamine (LPE), phosphatidylserine (PS), phosphoinositides (PI) and sphingomyelins (SM).

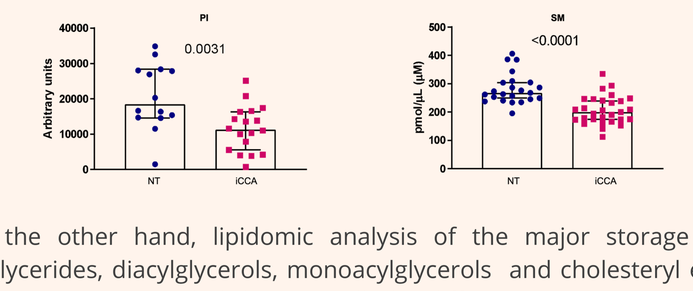


Cholesterol (Chol) accumulation also supports iCCA membrane building.



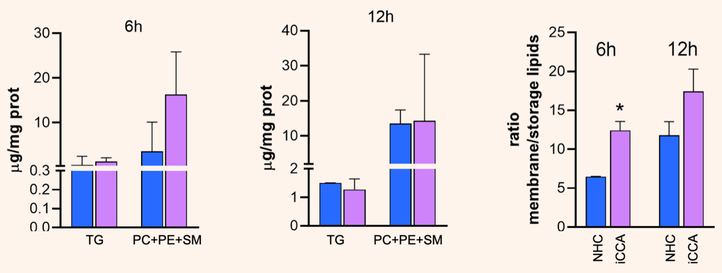
Conversely, **serum** content showed an **opposite trend**.



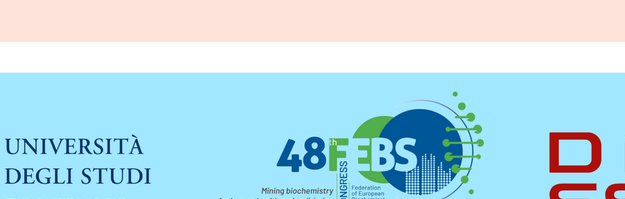


On the other hand, lipidomic analysis of the major storage lipids (triglycerides, diacylglycerols, monoacylglycerols and cholesteryl esters) in iCCA tissues revealed that, overall, the differences were considerably less significant than in NT controls.
Serum levels of TG and CE were unchanged.

The latter results were also **confirmed** through a **quantitative metabolic flux investigation** on NHC cell cultures and primary iCCA cell cultures. In both cell lines the palmitic acid d31 (PAD31) was predominantly incorporated in membrane lipids but, interestingly, in tumor cells the PAD31 was accumulated mostly.



In the SPL class, ceramide (Cer) and its downstream metabolite sphingosine-1-phosphate (S1P) are the main bioactive lipids, the latter acting as pro-survival and pro-proliferative mediators.
S1P was **decreased in iCCA tissue**.
In **iCCA patients' serum**, it was about **50% less expressed.**



Expression levels of SPHK1, the major enzyme of S1P synthetic pathway, were upregulated.
Conversely, Sphingosine-1-Phosphate Phosphatase 1 (SGPP1), which transiently de-phosphorylates S1P back to spingosine (Sph), and the Sphingosine-1-Phosphate Lyase 1 (SGPL1) which irreversibly cleaves S1P, were unchanged.
As for S1P extrusion from the cells, the transcript amount of the two transporters, the ATP Binding Cassette Subfamily C Member 1 (ABCC1) and the SPNS Lysolipid Transporter 2 Sphingosine-1-Phosphate (SPNS2), were both increased in iCCA tissue.
In iCCA tissue, S1PR3 transcripts were higher than in NT tissue.

Discussion

Overall **accumulation of lipids**:

- Acylcarnitine (CAR)
- Cholesteryl esters (CE)
- Sterols (ST)
- Ether-phospholipids (EtherPL)
- Glycosphingolipids (GSPL)
- Fatty acid (FA)

↑ + 65%

Alterations in FA metabolic pathways:

- ↑ *De novo* lipogenesis
- ↑ Membrane biogenesis (fluxomic)
- ↓ Membrane-associated transporters
- ↓ β-oxidation
- ↓ Lipid droplet

Conclusions

This study provides evidence in support of FA accumulation being dependent upon **impairment of β-oxidation**.
Furthermore, FAs prompt iCCA aggressiveness by **supporting membrane biogenesis** and the **generation of bioactive lipids** that boost proliferation.

Overall, these data suggest that targeting FA metabolism could represent a **viable innovative therapeutic intervention for iCCA**.

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