



Proteomics – a new approach to identify targets and biomarkers for hepatocellular carcinoma?

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Primary liver cancer, hepatocellular carcinoma (HCC), remains a global medical burden. Incidence rates are still high due to high prevalence of chronic viral hepatitis and the increasing numbers of obese and diabetic patients that develop non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) which are nowadays considered major drivers for HCC development (1,2). Treatment options for advanced disease stages are still limited and overall prognosis of HCC remains dismal, although immune checkpoint inhibitors seem to hold some promise over the previously established tyrosine kinase inhibitors (3).

Finding the right drug for the individual patient is therefore crucial for treatment success and biomarkers play a central role here. Considerable effort was put into finding genetic drivers for HCC that could represent novel and more potent drug targets and indeed, several mutations and genetic alterations have been identified, including TP53, hTERT or β -catenin (4). As HCC usually develops on the basis of other underlying chronic liver diseases, no clear genetic driver has been identified so far that was confirmed across the different etiologies. Furthermore, genetic alterations do not always translate into altered gene expression or protein functions. Therefore, also epigenetic regulators, gene expression profiling and miRNAs were investigated but also with these techniques no easily druggable targets were identified for HCC yet (5). Besides finding new targets, the need to identify predictive biomarkers for HCC is still high (6). As molecular targeted therapies are dependent on expression of target proteins,

proteomic technologies could be an interesting approach to overcome this hurdle.

Recently, Jiang *et al.* published results from a translational proteomics study using paired cancer and normal tissue samples from 110 early-stage HCC patients from China (7). Three distinct proteomic subsets of HCC were identified here: S-I with hepatocyte-like characteristics, S-II with additional proliferative characteristics and S-III that lacks the hepatocyte-like signature but has additional properties associated with metastasis and immune evasion. The S-III subset is clinically characterized by younger age and high degree of vascular invasion and AFP above 200 ng/mL with an overall poorer survival and higher recurrence rate than tumors in the other subsets. This phenotype was supported by proteomics findings of previously established pathways like upregulation of TGF, HIF1 or cell-cell or cell-matrix signaling components like integrins or Rho GTPases. In this subset, alterations in metabolic pathways were also detected and high expression of sterol O-acetyltransferase 1 (SOAT1 = Acyl-CoA:cholesterol acyltransferase 1, ACAT1) was associated with poor survival, increased tumor cell proliferation and enhanced invasion and metastasis. Analysis of other data bases confirmed the negative effects of high SOAT1 in HCC and other cancers although so far no reports on a prognostic role in HCC were available. Interestingly, SOAT1/ACAT1 protein expression show only poor concordance with mRNA expression, corroborating the functional relevance of a proteomics approach in identifying prognostic biomarkers as well as novel drug

targets. The role of SOAT1/ACAT1 was also functionally confirmed by shRNA-mediated downregulation of SOAT1/ACAT1 *in vitro* and pharmacological inhibition of the cholesterol synthesis pathway using the ACAT inhibitor avasimibe in xenograft models *in vivo*.

The strength of this study lies in a thorough multi-omics approach that included proteomics, phosphoproteomics and whole-genome sequencing in a training set and in a validation set of paired HCC tissue samples. Results were nicely validated using clinical data, bioinformatics analysis of other databases and functional experiments in cell culture and in patient derived xenograft *in vivo* models, thus representing a state-of-the-art approach to target identification. Yet, some points need to be critically considered when interpreting the presented data.

The samples selected for the analyses were obtained from HBV-driven early-stage HCC patients only. As the study population is homogeneous, the investigated sample size still seems adequate. While HBV still represents a major cause for HCC pathogenesis, esp. in Asian countries (8), patients with the here selected early-stage HCC may not be those with highest unmet medical need. In this study, patients with Barcelona Clinic Liver Cancer (BCLC) stages 0 and A were enrolled, which represent approx. 30% of all HCCs. For these patients, surgical resection, orthotopic liver transplantation or locoregional approaches like percutaneous ethanol injection (PEI) or radiofrequency ablation (RFA) are curative treatment options, leading to 5-year survival rates of 50–70% (9).

The liver is the primary metabolic organ of the body and responsible for cholesterol synthesis and modifications. Alterations in these metabolic pathways have been described previously during chronic liver disease and experimental data also indicate cholesterol-lowering drugs like statins could inhibit HCC formation and progression (10–12). In humans, the use of statins was associated with a reduced mortality in a retrospective analysis of a non-Asian population with only low HBV prevalence (8.0%) but high HCV prevalence (54.1%) (13). Further prospective and randomized trials are still needed in this setting. The proteomics analysis by Jiang *et al.* also demonstrated alterations in several components of the cholesterol metabolism, e.g., upregulation of CYP7A1 or LDL receptor [extended data in (7)]. Interestingly, these components of cholesterol metabolism are also under the control of fibroblast growth factors (esp. FGF15 and FGF21) (14), and it would be interesting to understand whether also these mediators were differentially expressed in early-stage

HCC, as FGF ligands and FGF receptors are commonly upregulated in HCC and associated with poorer survival and resistance to therapy (15,16). Interestingly, also HBV has been shown to affect lipid and cholesterol metabolism. In HBV transgenic mice, transcriptional upregulation of e.g., sterol regulatory element binding protein 2 (SREBP2) was demonstrated (17), similar to the findings by Jiang *et al.* Additional regulation of cholesterol metabolism by miRNAs was recently also demonstrated in a small subset of Japanese patient samples with HBV infection (18). Thus, further functional studies are needed to study the interplay and interdependencies of cholesterol metabolism, cell proliferation, inflammation and HBV (and other etiologic co-factors) to clarify its role in HCC pathogenesis and validate SOAT1/ACAT1 as a novel drug target.

Considerable progress has been made in the past decade to improve proteomics technologies and opened the possibilities to differentiate even at the subcellular or subproteome level and for various posttranslational protein modifications like glycosylation or phosphorylation (19). Besides technical challenges linked to sample preparation and handling, the heterogeneity of tumors is seen as a limitation of proteomics in cancer drug discovery. Large sample sets are needed to generate biologically meaningful data that can be separated from background noise, which may require validation of the above discussed data by larger consortia. Availability of tissue is also a limiting factor as practice guidelines only recently started to recommend taking biopsies from HCC patients (20). Liquid biopsies therefore represent an interesting option to overcome this bottleneck (21,22). Besides genomic analyses, proteomic profiling can be performed from human plasma or serum samples, leading to the identification of several prognostic and diagnostic biomarkers for HCC (23,24).

In summary, the study by Jiang *et al.* nicely demonstrated the power of a multi-omics approach to identify potential new drug targets and biomarkers in HCC. Although the patient number is not too high, the homogenous population allowed identifying changes in cholesterol metabolism mediated by increased expression of SOAT1/ACAT1. The translational approaches using a pharmacological inhibitor or shRNA knockdown indicate that this target should be further explored. If inhibition of SOAT1/ACAT1 will be superior to currently established therapy for early-stage HCC only in HBV positive patients or in general needs to be explored in future randomized and controlled trials with proper patient selection. Further validation of the target also in later disease stages is also warranted as treatment options are still limited for these patients.

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Footnote

Conflicts of Interest: The author is employee and shareholder of Bayer AG, Germany.

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