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LIQUID BIOPSY PROTEINS AS PSC-SPECIFIC AND PAN-CCA BIOMARKERS OF CANCER RISK, EARLY DIAGNOSIS AND SURVIVAL MIRRORING TUMOR CELLS

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Resumen

Introduction and objectives: Cholangiocarcinomas (CCAs), heterogeneous biliary tumors with

dismal prognosis, lack accurate early-diagnostic methods, especially important for individuals at high-risk (i.e., primary sclerosing cholangitis (PSC)). Here, we searched for protein biomarkers in serum extracellular vesicles (EVs).

Methods: EVs from patients with isolated PSC (n = 45), concomitant PSC-CCA (n = 42), PSC who developed CCA during follow-up (PSC to CCA; n = 25), CCAs from non-PSC etiology (n = 56), hepatocellular carcinoma (n = 34) and healthy individuals (n = 55) were characterized by mass-spectrometry. Diagnostic biomarkers of PSC-CCA, non-PSC CCA or CCAs regardless etiology (pan-CCAs) were defined, and their expression was evaluated in human organs/tissues and within CCA tumors at single-cell level. Prognostic EV-biomarkers for CCA were investigated.

Results: High-throughput proteomics identified candidate diagnostic biomarkers for PSC-CCA, non-PSC CCA or pan-CCA, as well as and for differential diagnosis of intrahepatic CCA and HCC, that were cross-validated by ELISA using total serum. Machine learning logit modelling disclosed CRP/FRIL/Fibrinogen algorithm with diagnostic value for early-stage PSC-CCA v s isolated PSC (AUC = 0.944; OR = 82.0), overpowering CA19-9 (AUC = 0.735; OR = 9.3). An algorithm combining CRP/VWF/PIGR//Fibrinogen allowed the diagnosis of early-stage non-PSC CCAs compared to healthy individuals (AUC = 0.999; OR = 1,115). Noteworthy, levels of Fibrinogen/CRP/PIGR/FRIL showed predictive capacity for CCA development in patients with PSC before clinical evidences of malignancy. Multi-organ transcriptomic analysis revealed that serum EVbiomarkers were mostly expressed in hepatobiliary tissues, and scRNA-seq and immunofluorescence analysis of CCA tumors showed their presence mainly in malignant cholangiocytes. Multivariable analysis unveiled EV-prognostic biomarkers independent to clinical features, with COMP/GNAI2/CFAI and ACTN1/MYCT1/PF4V associated negatively or positively to patients' survival, respectively.

Conclusions: Serum EVs contain protein biomarkers for the prediction, early diagnosis and prognosis estimation of CCA, representing a novel tumor cell-derived liquid biopsy for personalized medicine.