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Plasma-derived candidate biomarkers for detection of gallbladder carcinoma

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Gallbladder carcinoma (GBC) is a major cancer of the gastrointestinal tract with poor prognosis. Reliable and affordable biomarker-based assays with high sensitivity and specificity for the detection of this cancer are a clinical need. With the aim of studying the potential of the plasma-derived extracellular vesicles (EVs), we carried out quantitative proteomic analysis of the EV proteins, using three types of controls and various stages of the disease, which led to the identification of 86 proteins with altered abundance. These include 29 proteins unique to early stage, 44 unique to the advanced stage and 13 proteins being common to both the stages. Many proteins are functionally relevant to the tumor condition or have been also known to be differentially expressed in GBC tissues. Several of them are also present in the plasma in free state. Clinical verification of three tumor-associated proteins with elevated levels in comparison to all the three control types—5'-nucleotidase isoform 2 (NT5E), aminopeptidase N (ANPEP) and neprilysin (MME) was carried out using individual plasma samples from early or advanced stage GBC. Sensitivity and specificity assessment based on receiver operating characteristic (ROC) analysis indicated a significant association of NT5E and ANPEP with advanced stage GBC and MME with early stage GBC. These and other proteins identified in the study may be potentially useful for developing new diagnostics for GBC.

Abbreviations

GBC	Gallbladder carcinoma
EVs	Extracellular vesicles
GSD	Gallstone disease
XGC	Xanthogranulomatous cholecystitis
iTRAQ	Isobaric tags for relative and absolute quantitation
ELISA	Enzyme-linked immunosorbent assay
MME	Neprilysin
NT5E	5'-Nucleotidase isoform 2
ANPEP	Aminopeptidase N
ROC	Receiver operating characteristic curve
AUC	Area under the ROC curve
NTA	Nanoparticle tracking analysis
SCX	Strong cation exchange
LC-MS	Liquid chromatography-mass spectrometry
FDR	False discovery rate
PSMs	Peptide spectral matches
STRING	Search tool for the retrieval of interacting genes/proteins

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