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Immunoproteomics approach revealed elevated autoantibody levels against ANXA1 in early stage gallbladder carcinoma



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Abstract

Background: Early diagnosis is important for the timely treatment of gallbladder carcinoma (GBC) patients and may lead to increased survival outcomes. Here, we have applied serological proteome analysis (SERPA), an immunoproteomics approach, for the detection of ‘tumor-associated antigens (TAAs) that elicit humoral response’ in early stage GBC patients.

Methods: Total protein from pooled tumor tissue of GBC patients ($n = 7$) was resolved by two-dimensional gel electrophoresis (2-DE) followed by immunoblotting using pooled blood plasma from healthy volunteers ($n = 11$) or gallstone disease (GSD) cases ($n = 11$) or early stage GBC (Stage I and II) ($n = 5$) or GBC stage IIIA ($n = 9$). 2-D gel and immunoblot images were acquired and analyzed using PDQuest software to identify immunoreactive spots in GBC cases in comparison to controls. Proteins from immunoreactive spots were identified by liquid chromatography-tandem mass spectrometric analysis (LC-MS/MS). Autoantibody levels for two of the functionally relevant proteins were investigated in individual plasma samples (52 cases and 89 controls) by dot blot assay using recombinant proteins.

Results: Image analysis using PDQuest software identified 25 protein spots with significantly high or specific immunoreactivity in GBC cases. Mass spectrometric analysis of 8 corresponding protein spots showing intense immunoreactivity (based on densitometric analysis) in early stage GBC or GBC stage IIIA cases led to the identification of 27 proteins. Some of the identified proteins include ANXA1, HSPD1, CA1, CA2, ALDOA and CTSD. Among the two proteins, namely ANXA1 and HSPD1 verified using a cohort of samples, significantly elevated autoantibody levels against ANXA1 were observed in early stage GBC cases in comparison to healthy volunteers or GSD cases (unpaired t-test, $p < 0.05$). Receiver operating characteristic (ROC) curve analysis for ANXA1 showed an Area under the Curve (AUC) of 0.69, with 41.7% sensitivity against a specificity of 89.9% for early stage GBC. IHC analysis for ANXA1 protein showed ‘high’ expression levels in 72% of GBC cases whereas all the controls showed ‘low’ expression levels.

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