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Bile proteomics for discovery of biomarkers to differentiate between gallbladder polyp and gallstone

Hyunesoo Kwon[1], Young Eun Kim[2], Hoeil Chung[3], Dukjin Kang[2] and Tae-Young Kim[1*]

Abstract

Gallbladder polyps and gallstones, despite having distinct pathological mechanisms, present similar clinical features that challenge accurate diagnosis and timely intervention. This study aimed to identify potential diagnostic biomarkers for distinguishing between these conditions through a comprehensive proteomic analysis of bile juice. Bile samples from patients with gallbladder polyps, gallstones, and healthy controls were analyzed using nano-liquid chromatography-tandem mass spectrometry. A total of 167 proteins were identified in polyp samples and 118 in gallstone samples, with 99 proteins shared between the two groups. Comparative analysis revealed 14 proteins significantly upregulated and 15 downregulated in gallbladder polyp samples. Gene Ontology and KEGG pathway analyses revealed that upregulated proteins in polyps were primarily involved in oxidative stress and metabolic processes, while downregulated proteins were associated with extracellular matrix organization. Most notably, hemoglobin subunits HBA1 and HBB showed significant elevation in polyps, indicating enhanced oxygen transport and inflammation, while extracellular matrix proteins LUM and EZR showed reduced expression, suggesting compromised structural integrity. These findings underscore the potential of differentially expressed proteins in bile as diagnostic biomarkers, offering a promising avenue for early and accurate differentiation between gallbladder polyps and gallstones.

Keywords Bile juice, Proteomics, Gallbladder polyp, Gallstone, Biomarker

Introduction

Gallbladder polyps and gallstones are notable hepatobiliary diseases due to their potential to cause severe health issues such as gallbladder cancer and acute cholecystitis, respectively (Nuño-Guzmán et al. 2016; Matos et al. 1992). According to previous studies, 5.9% of gallbladder polyps have a chance to progress to gallbladder cancer (Martin et al. 2018) and 20% of gallstones can result in acute cholecystitis (Thadikkaran et al. 2005). Prompt early identification of gallbladder polyps or gallstones, coupled with targeted treatment for each hepatobiliary disease, can effectively prevent or postpone the development of related health complications. Distinct differences in the underlying causes and medical interventions for

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gallbladder polyps and gallstones (Liu et al. 2006) necessitates a reliable diagnostic tool for early differentiation between two hepatobiliary diseases. Currently, there is a lack of reliable diagnostic method for distinguishing gallbladder polyps and gallstones in their early stages.

Ultrasonography and computed tomography (CT) are commonly used imaging techniques for detection of hepatobiliary diseases (Swelm et al. 2014). However, distinguishing between gallbladder polyps and gallstones smaller than 5 mm using conventional imaging techniques is challenging due to their similar appearance (Dierks et al. 2019). The presence of mucosal folds, bile sludge, and tiny gallstones can often obscure the visual differentiation between gallbladder polyps and cholesteric gallstones with ultrasonography (LaRusso 1984). CT scans offer superior precision and reduced false positives compared to ultrasound in diagnosing hepatobiliary diseases. However, their effectiveness is limited to polyps larger than 10 mm, hindering early detection (Abramson 2008). Conventional imaging techniques often face challenges in distinguishing gallbladder polyps from gallstones, as these methods primarily depend on visual characteristics such as shape and size. To overcome the limitations of current diagnostic method, the development of novel approaches for the early and accurate differentiation of gallbladder polyps and gallstones is imperative.

Biomarkers serve as critical indicators of health and disease, enabling early detection through the analysis of specific molecules or molecular patterns within biological samples (Oleson and Corbett 2018). They can detect subtle physiological changes indicative of disease onset, often before symptoms manifest. A diverse set of biomarkers has been detected in different biofluids for hepatocellular carcinoma (HCC). For instance, alpha-fetoprotein in blood (Chang et al. 2024) and 8-oxodeoxyguanosine in urine (Hayes et al. 2005), have shown potential for early HCC diagnosis. Similarly, endocrine-disrupting chemical biomarkers have been effectively measured in human saliva using GC–MS/MS, demonstrating the feasibility of non-traditional biofluids as diagnostic matrices (Vu et al. 2020). Furthermore, circulating tumor DNA in blood has been linked to the progression of HCC, offering valuable insights for early therapeutic actions. Biomarkers are also highly specific and can distinguish between diseases with similar symptoms but different underlying biological processes. Han et al. demonstrated that neuropeptides such as Substance P and Neuropeptide Y could serve as potential diagnostic biomarkers for acute myocardial infarction, highlighting their clinical relevance in differentiating cardiovascular diseases with overlapping symptoms (Han et al. 2014). Despite the ease of collection, body fluids like blood and urine often exhibit limitations in sensitivity and specificity for biomarker research (Bretscher et al. 2002).

Bile juice, a direct secretion from the liver and biliary system, offers a promising alternative to blood and urine for biomarker research in hepatobiliary diseases. Unlike other biofluids, bile juice contains higher concentrations of hepatobiliary disease-specific biomarkers, enhancing its diagnostic potential (Portincasa et al. 2006). This diverse biomarker profile, encompassing proteins (Zhu et al. 2008), metabolites, and nucleic acids (Ruf and Mueller 2006), enables more precise disease detection and monitoring, overcoming the limitations of traditional biomarkers found in blood and other body fluids. Importantly, our study focuses on analyzing bile fluid—not the crystalline gallstone structures themselves—as bile directly reflects the pathophysiological state of the gallbladder and biliary system (Kristiansen et al. 2004). The bile proteomes of patients with gallbladder polyps and gallstones provide a clinically relevant framework for comparison, as gallstones represent the most common indication for cholecystectomy and the primary differential diagnosis for polyps in clinical practice (Yang et al. 2023). Both conditions involve alterations in bile composition and flow dynamics, yet they arise from distinct pathological mechanisms that may be reflected in differential protein expression patterns. Characterizing these distinct bile proteomic signatures may therefore yield important insights for differential diagnosis and illuminate underlying disease mechanisms that could inform therapeutic strategies.

This study aims to identify distinct protein expression patterns in bile samples from patients with gallbladder polyps and gallstones, compared to healthy individuals. By employing quantitative proteomics analysis using nanoflow liquid chromatography-tandem mass spectrometry (nLC-MS/MS), we seek to uncover biomarkers that differentiate between these two hepatobiliary diseases, ultimately enhancing the accuracy of disease diagnosis.

Results

Differential protein expression profiles between gallbladder polyps and gallstones

A proteomic analysis of gallbladder polyp and gallstone samples identified 167 (Table S1) and 118 proteins (Table S2), respectively, with 99 shared proteins representing core functions in bile secretion and gallbladder physiology (Fig. 1A and Table S3). Distinct proteomic signatures were also evident, with 68 proteins unique to polyps and 19 unique to gallstones, highlighting condition-specific protein expression patterns.

Comparative analysis revealed significant differences in protein expression, with 14 proteins upregulated and 15 downregulated in the polyp group (fold change > 2 for

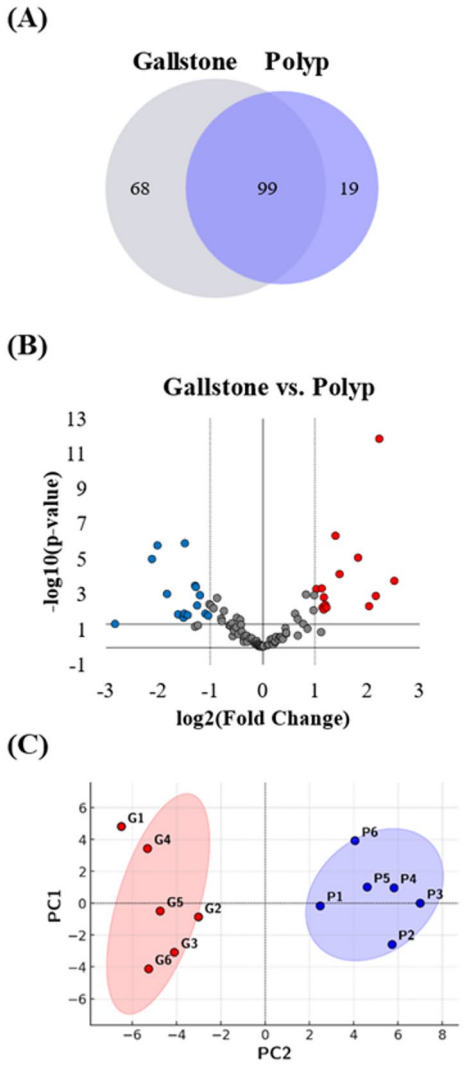

Fig. 1 (A) Venn diagram of identified proteins, (B) volcano plot, and (C) PCA analysis of differentially expressed proteins between polyp and gallstone samples

each). A volcano plot (Fig. 1B) illustrates these differentially expressed proteins, showing upregulated proteins in polyps (red) and gallstones (blue) as potential biomarkers for gallbladder disease progression. Principal component analysis (PCA) further demonstrated clear separation between polyp and gallstone samples along the first principal component (Fig. 1C), confirming distinct proteomic signatures between the two conditions.

Functional enrichment analysis of differentially expressed proteins

To elucidate the biological implications of the differentially expressed proteins, Gene Ontology (GO) enrichment analysis was performed to identify significantly enriched biological processes, cellular components, and molecular functions associated with proteins upregulated (Fig. 2A) or downregulated (Fig. 2B) in gallbladder polyp samples compared to gallstone samples.

Upregulated proteins in gallbladder polyps showed significant enrichment in biological processes related to gas transport, reactive oxygen species metabolism, and regulation of coagulation. These findings suggest increased oxidative stress and metabolic adaptations in polyps. Enriched cellular components included the extracellular exosome, extracellular space, and collagen-containing extracellular matrix, indicating active remodeling of the extracellular environment. At the molecular function level, enriched pathways encompassed oxygen carrier activity, antioxidant activity, and oxidoreductase activity, with proteins such as HSP90AA1 and GAPDH playing central roles in these processes.

Downregulated proteins in gallbladder polyps were involved in biological processes such as negative regulation of fibrinolysis, plasminogen activation, and multicellular organismal processes. These proteins were enriched in cellular components including extracellular exosomes, the cell surface, and the collagen-containing extracellular matrix, suggesting potential disruption of extracellular matrix (ECM) structure and signaling. Molecular functions associated with these downregulated proteins included endopeptidase inhibitor activity, enzyme binding, and chaperone binding. Notably, ECM organization and cytoskeletal stability proteins, including LUM and EZR, were downregulated, highlighting diminished structural support within the polyp ECM compared to gallstones.

Protein expression patterns in gallbladder polyp and gallstone samples

Expression patterns of differentially expressed proteins between gallbladder polyp and gallstone samples were visualized using heatmap analysis (Fig. 3). The heatmap demonstrates clear distinction between the two conditions, with blue representing lower expression and red indicating higher expression. Proteins are grouped based on expression pattern similarities, while samples are arranged sequentially from G1-G6 (gallstone samples) and P1-P6 (polyp samples).

Cluster analysis revealed two distinct protein groupings based on their expression patterns in polyp samples relative to gallstone samples. Cluster 1 comprises proteins significantly upregulated in polyp samples, showing enrichment in nitrogen metabolism and African trypanosomiasis pathways (adjusted $p < 0.05$). This cluster includes key proteins such as HBB, HBA1, and TKT, which are involved in oxygen transport and metabolic adaptations, indicating altered metabolic activity in polyp tissues. Cluster 2 contains proteins significantly downregulated in polyp samples, with enrichment in proteoglycan-related pathways (adjusted $p < 0.05$). Notable

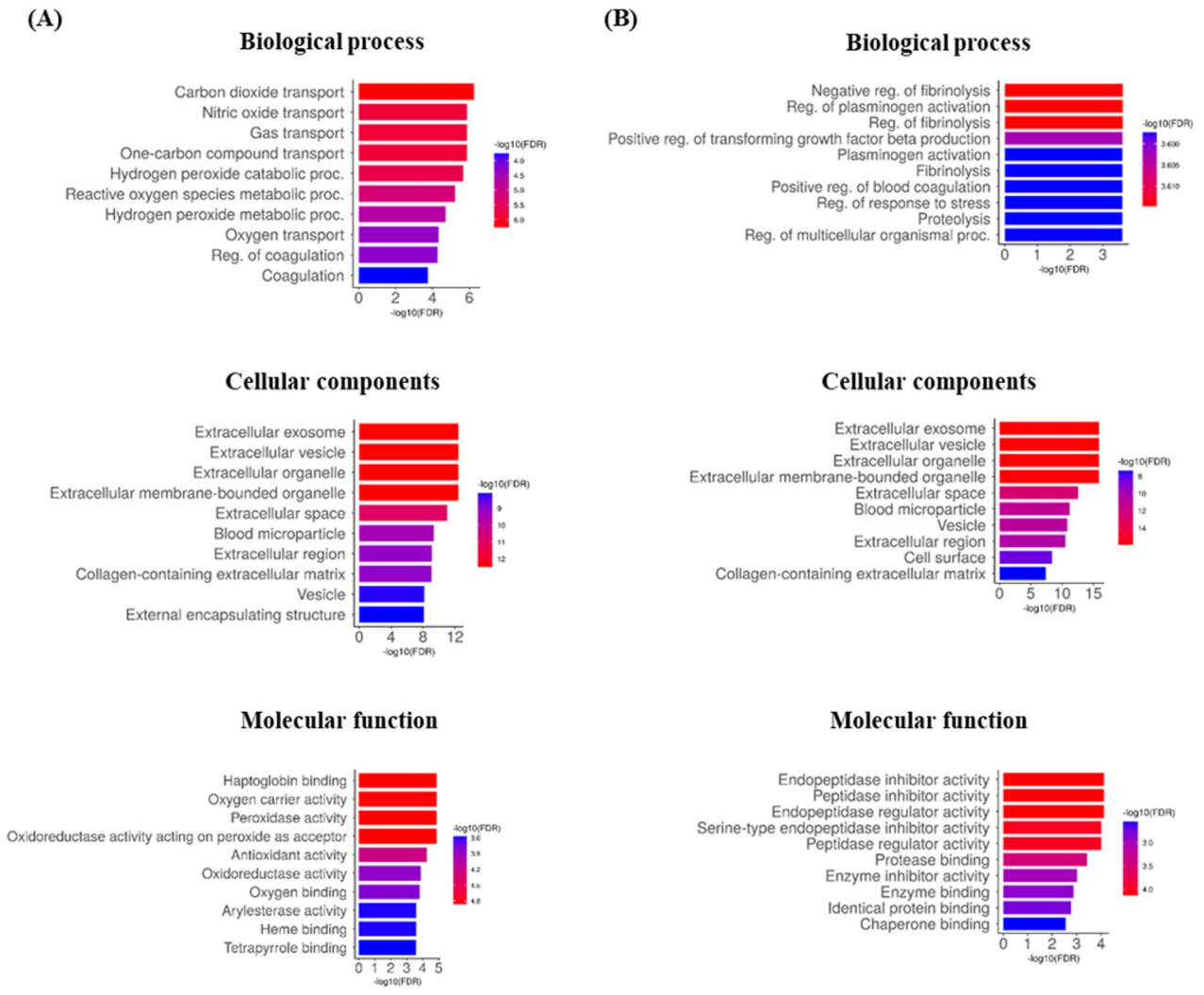

Fig. 2 Gene Ontology (GO) enrichment analysis of (**A**) upregulated and (**B**) downregulated proteins in polyp tissues compared to gallstones. The color gradient in all panels represents the significance of enrichment (– log10(FDR))

proteins in this cluster include LUM and EZR, which are crucial for ECM organization and cytoskeletal integrity. Their reduced expression suggests compromised structural and functional integrity in the ECM of polyp tissues.

Discussion

This study highlights distinct protein expression profiles in gallbladder polyps and gallstones, offering valuable insights into the molecular mechanisms driving these conditions. Comparative proteomic analysis identified a total of 14 significantly upregulated and 15 significantly downregulated proteins in gallbladder polyp samples relative to gallstone samples, as illustrated in the volcano plot (Fig. 1B). To identify biologically coherent expression patterns within these differentially expressed proteins (DEPs), we performed unsupervised hierarchical clustering analysis, which revealed two distinct protein clusters with consistent expression trends across samples: 8 upregulated and 7 downregulated proteins (Fig. 3). The expression changes of these cluster-defining DEPs are further visualized as box plots in Fig. 4, confirming their robust and consistent differential regulation between gallbladder polyp and gallstone groups. These differential expression patterns underscore the unique pathological processes underlying polyp development and identify potential biomarkers for diagnostic purposes.

Among the upregulated proteins in gallbladder polyps, AKR1C1, CA1, CYB5A, GAPDH, HBA1, HBB, HSP90AA1, and TKT emerged as key players in diverse cellular processes. AKR1C1 facilitates detoxification and metabolic regulation through oxidoreductase activity (Endo et al. 2019), while CA1 supports pH balance and ion transport within cells (Neckers and Workman 2012). CYB5A influences electron transfer across multiple metabolic pathways (Bai et al. 2015), and GAPDH is integral to glycolysis, underscoring the metabolic

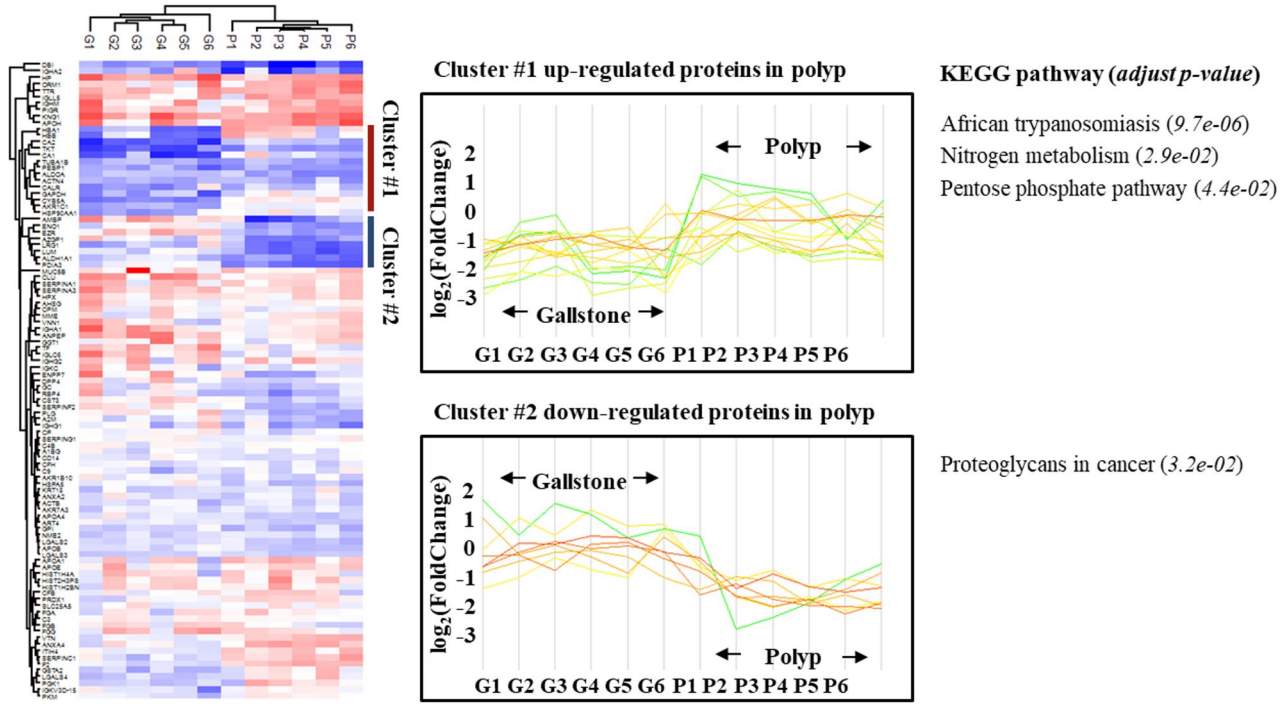

Fig. 3 Heatmap of the 99 shared proteins identified in both in gallbladder polyp and gallstone samples. Color intensity represents relative expression levels (blue, lower; red, higher). Proteins and samples are clustered based on expression similarity. The heatmap distinguishes distinct protein expression patterns between polyps and gallstones, revealing clusters of differentially expressed proteins. The top and bottom bar plots in the middle display the log2-fold change of protein clusters in polyps relative to gallstones and in gallstones relative to polyps, respectively. KEGG pathway analysis was performed using the proteins included in each cluster

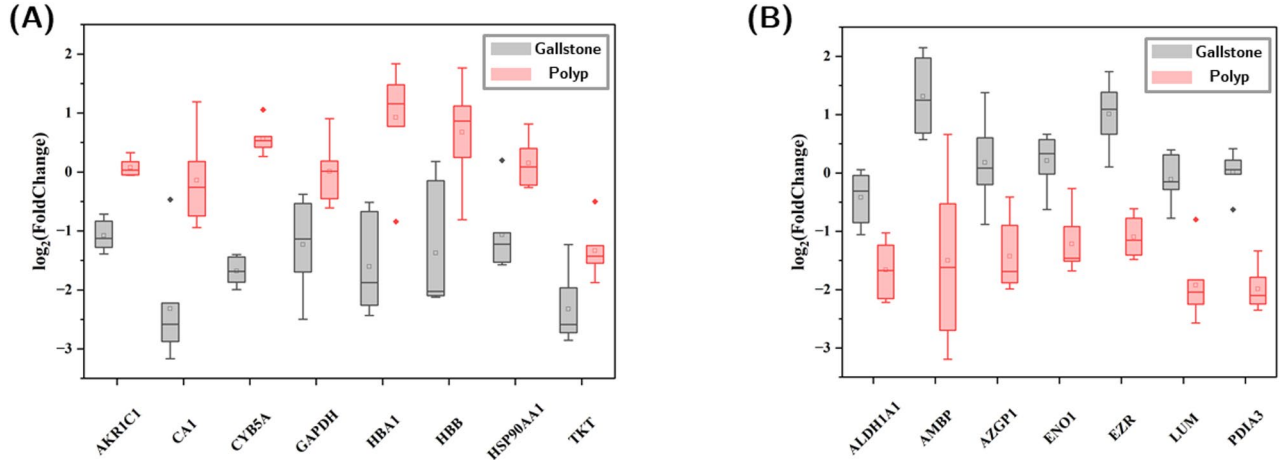

Fig. 4 Box and whisker plots showing the log2-fold change of significantly ($p < 0.05$) differentially expressed proteins between gallbladder polyp and gallstone samples: (A) 8 upregulated proteins in polyps and (B) 7 downregulated proteins in polyps

adaptations in polyps (Lazarev et al. 2020). Notably, the hemoglobin subunits HBA1 and HBB, which mediate oxygen transport (Filser et al. 2022), were significantly upregulated. These findings are particularly intriguing given their involvement in nitric oxide (NO) transport, a critical signaling molecule in inflammation (Tripathi et al. 2007). The elevated expression of HBA1 and HBB suggests enhanced NO transport capacity in gallbladder polyps, potentially contributing to the chronic inflammatory milieu that promotes polyp growth (Chang et al. 2024). This chronic inflammation, driven by excessive NO signaling, may lead to tissue damage and further facilitate polyp progression, positioning these proteins as promising biomarkers for diagnosis.

Conversely, the downregulated proteins in gallbladder polyps, including ALDH1A1, AMBP, AZGP1, ENO1, EZR, LUM, and PDIA3, are associated with essential functions such as detoxification (ALDH1A1) (Tomita

et al. 2016), protease inhibition (AMBP) (Pethő et al. 2021), lipid mobilization (AZGP1) (Faulconnier et al. 2019), glycolysis (ENO1) (Zhou et al. 2019), cytoskeletal organization (EZR) (Xu and Zhang 2021), extracellular matrix organization (LUM) (McEwan et al. 2006), and protein folding and disulfide bond formation (PDIA3) (Mahmood et al. 2021). Notably, the reduced expression of EZR—a key regulator of cytoskeletal integrity and glucose metabolism (Bretscher et al. 2002)— indicates potential perturbations in bile composition and flow dynamics, fundamental factors in the pathogenesis of both polyps and gallstones. Such alterations may further compromise gallbladder homeostasis, contributing to disease progression. In contrast, gallstone samples were enriched for pathways related to proteoglycans in cancer and HIF-1 signaling, reflecting their hypoxic microenvironment and ECM remodeling. These findings align with the role of gallstones in bile stagnation and altered bile composition, emphasizing the interplay between ECM organization and gallstone pathogenesis.

The identification of these differentially expressed proteins presents significant implications for diagnostic and therapeutic interventions. The elevated hemoglobin subunits HBA1 and HBB exhibit potential utility as non-invasive biomarkers, facilitating early detection of gallbladder polyps through serological or biliary analysis. Furthermore, the established relationship between NO signaling and inflammatory processes in polyp development presents a promising therapeutic target for modulating the inflammatory microenvironment and attenuating disease progression (Sadek et al. 2019). Additionally, therapeutic strategies aimed at restoring EZR function or targeting its downstream effectors may ameliorate bile composition and flow dynamics, potentially reducing the risk of gallbladder pathologies. The observed proteomic divergence between gallbladder polyps and gallstones warrants further investigation into their shared and distinct molecular mechanisms. Given that both conditions manifest alterations in bile composition and flow dynamics, the differential protein expression patterns observed in polyps may have reciprocal implications for gallstone formation. This interrelationship emphasizes the necessity for comprehensive management strategies addressing both the overlapping and unique aspects of these hepatobiliary disorders.

We acknowledge that the number of proteins identified in this study (167 in polyps and 118 in gallstones) is modest compared to typical tissue or cell line proteomics studies, which routinely identify thousands of proteins. However, this comparison may not be entirely appropriate given the fundamental differences between bile and tissue proteomes. Bile presents unique analytical challenges as a biofluid characterized by low total protein content, extreme dynamic range dominated by high-abundant proteins, and limited clinical sample volumes that constrain extensive fractionation approaches (Farina et al. 2009a, b). Importantly, our protein identification numbers align well with previous bile proteomics literature. Kristiansen et al. identified 87 unique proteins in human bile (Kristiansen et al. 2004), while Ciordia et al. emphasized the persistent challenge of low protein abundance in bile even under optimized analytical conditions (Ciordia et al. 2021). In this context, our identification of approximately 100–200 proteins per condition is consistent with the expected coverage for non-depleted, non-fractionated bile proteomics and provides exploratory yet biologically meaningful insights into gallbladder disease mechanisms.

This limited proteome depth partly reflects the absence of high-abundance protein depletion or peptide fractionation steps in our workflow, as dominant proteins such as albumin and immunoglobulins can obscure the detection of lower-abundance species (Farina et al. 2009a, b). Future investigations should integrate advanced enrichment strategies to enhance proteome depth, including high-abundance protein depletion, peptide fractionation, or advanced MS acquisition methods. Such approaches will not only expand proteome coverage but also strengthen confidence in the biomarker candidates identified in this exploratory study.

A limitation of this study is the inability to perform individual statistical comparisons with healthy controls due to insufficient protein yields from non-diseased bile samples. The four healthy bile samples were pooled to serve as a normalization reference, which enabled robust quantification of polyp versus gallstone differences but precluded direct assessment of disease-specific versus shared alterations relative to healthy baseline. Future studies with larger bile collection volumes from healthy donors will be essential to establish a comprehensive baseline bile proteome and to distinguish disease-specific markers from general gallbladder pathology signatures.

Conclusions

This investigation presents a comprehensive proteomic analysis of gallbladder polyp and gallstone samples using nano-LC-MS/MS, revealing 167 distinct proteins in polyps and 118 in gallstones, with 99 proteins shared between conditions. Differential expression analysis identified 14 upregulated and 15 downregulated proteins in polyps, while PCA demonstrated clear separation between polyp and gallstone samples, establishing distinct proteomic signatures. GO and biological process enrichment analyses elucidated key pathways in polyp and gallstone formation, highlighting significant roles for inflammatory processes, nitric oxide transport, and metabolic pathways.

Several proteins emerged as promising biomarker candidates for gallbladder polyp diagnosis, particularly HBA1, HBB, and EZR. These proteins, which regulate NO transport and cytoskeletal integrity, provide novel insights into the molecular mechanisms underlying polyp formation and inflammation. The findings suggest potential therapeutic interventions through targeting upregulated pathways involved in glycolysis and NO signaling to impede polyp growth, while strategies aimed at restoring EZR function may help maintain proper bile composition and mitigate the risk of gallbladder diseases.

Although these findings are promising, validation studies involving larger, independent cohorts are essential to confirm the diagnostic and therapeutic potential of these proteins. The identification of proteins implicated in both polyp and gallstone development underscores the importance of further investigating shared molecular pathways, potentially leading to more comprehensive management strategies for gallbladder diseases.

In conclusion, our study provides valuable insights into the molecular underpinnings of gallbladder polyp and gallstone formation. The identified proteins offer promising biomarkers and therapeutic targets, paving the way for improved diagnosis, treatment, and patient outcomes in gallbladder disease.

Materials and methods

Patients and clinical samples

A total of 16 bile juice samples were collected from 6 patients with GB polyps, 6 patients with GB gallstones, and 4 healthy individuals who underwent cholecystectomy at Hanyang University Hospital (Seoul, South Korea). The samples were immediately frozen to prevent degradation and stored in a deep freezer. Written informed consent was obtained from all participants, and the study was conducted in full accordance with the protocol approved by the Institutional Review Board of Hanyang University (HYI-18–227–3). All procedures were performed in accordance with the relevant guidelines and regulations.

Sample preparation for proteomic analysis

Bile samples were lysed in a lysis buffer containing 8 M urea (Sigma-Aldrich, St. Louis, MO, USA), 50 mM Tris-HCl (pH 8.0), 75 mM NaCl, and a protease inhibitor cocktail (Roche, West Sussex, UK). After sonication in 3 s pulses with 2 s pauses between pulses and centrifugation at 12,000 rpm for 15 min, the supernatant was collected for protein digestion. Protein concentrations were measured using the bicinchoninic acid assay to ensure equal amounts of protein for digestion. The proteins were reduced with 10 mM dithiothreitol for 2 h at 37 °C, followed by alkylation with 20 mM iodoacetamide (IAA) for 30 min in the dark at room temperature. Excess IAA was quenched with 40 mM L-cysteine for 30 min at room temperature. The sample was then diluted with 50 mM ammonium bicarbonate to reduce the urea concentration to less than 1 M for optimal trypsin activity. Proteins were digested with trypsin (Promega, Madison, WI, USA) at a ratio of 1:50 (w/w) for 18 h at 37 °C. Digestion was stopped by adding 1% formic acid (FA), and the peptides were desalted using a 10 mg OASIS HLB cartridge (Waters, MA, USA). The eluted peptides were then dried in a vacuum evaporator (HyperVAC-LITE, Gyrozen, South Korea) and resuspended in 0.1% FA.

nLC-MS/MS analysis

For nLC, two columns were prepared: a trapping column was a one-end blocked capillary tubing packed with C18 resin (5 μm, 200 Å) and a reverse-phase (RP) column was a 150 mm capillary filled with C18 resin (3 μm, 100 Å). Peptides were injected into the trapping column, followed by a 120 min RP gradient at a column flow rate of 200 nL/min. The mobile phase consisted of buffer A (0.1% FA in water) and buffer B (0.1% FA and 2% water in acetonitrile). The gradient was as follows: 2% B for 10 min, 2–10% B for 1 min, 10–17% B for 4 min, 17–33% B for 70 min, 33–90% B for 3 min, 90% B for 15 min, followed by a return to 2% B over 2 min, and then holding at 2% B for the final 15 min. The peptides were analyzed using a Q-Exactive FT orbitrap mass spectrometer (Thermo Fisher Scientific) in data-dependent mode. Full-scan MS spectra (m/z 300–1800) were acquired with an automatic gain control target of 3E6 at 70,000 resolution. The 12 most abundant ions were selected for HCD fragmentation with 27% normalized collision energy.

Database searches and quantification

Raw MS data files were searched against the UniProt human database (May 10, 2020) using the MaxQuant search engine (version 1.6.1.0, Max Planck Institute, Munich, Germany) for protein identification and relative quantification (Cox and Mann 2008). Search parameters included: two allowed missed cleavages, mass tolerances of 4.5 ppm and 20 ppm for precursor and fragment ions, carbamidomethylation of cysteine as a fixed modification, acetylation of the N-terminal residue and oxidation of methionine as variable modifications, and a false discovery rate of 0.01 for both peptides and proteins.

TMT labeling was performed using a 10-plex configuration according to the manufacturer's instructions (Thermo Fisher Scientific). Due to insufficient protein yield from individual healthy bile samples for robust MS analysis, the four normal bile samples were pooled and labeled with the TMT-126 reporter channel in both TMT sets. This pooled normal reference served as a common internal standard across experimental batches, enabling cross-batch normalization and direct quantitative

comparison between polyp and gallstone samples while minimizing inter-batch technical variation. Reporter ion intensities were $\log_2$-transformed and normalized relative to the pooled normal channel, followed by median centering to correct for loading differences and systematic bias.

For each protein, pairwise $\log_2$ fold change ($\log_2$FC) values were calculated, where P represents the polyp sample, G represents the gallstone sample, and C represents the pooled normal control. Specifically, $\log_2$FC was computed by subtracting the $\log_2$-transformed intensity of the control from that of the disease sample ($\log_2\text{FC} = \log_2(\text{P}) - \log_2(\text{C})$ or $\log_2(\text{G}) - \log_2(\text{C})$). Thus, six $\log_2$FC values were obtained per protein, and the mean $\log_2$FC together with the standard error of the mean (SEM) was calculated to summarize overall expression changes and variability. Missing values were imputed from a normal distribution (width = 0.3, downshift = 1.8) using Perseus (version 1.6.15.0) (Tyanova et al. 2016). Only proteins identified with at least two unique peptides—excluding contaminants and reverse hits—were retained for downstream analysis. Statistical significance was assessed using two-tailed paired Student's *t*-tests on the $\log_2$FC values, with significance defined as $p \le 0.05$. Proteins with $|\log_2\text{FC}| \ge 1$ and $p \le 0.05$ were considered differentially expressed. All statistical analyses were conducted using Perseus and Microsoft Excel. To ensure data reliability, only proteins consistently quantified across three biological replicates were included in the final dataset.

Abbreviations

CT	Computed tomography
ECM	Extracellular matrix
FA	Formic acid
GO	Gene Ontology
HCC	Hepatocellular carcinoma
IAA	Iodoacetamide
NO	Nitric oxide
PCA	Principal component analysis
RP	Reversed-phase

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s40543-025-00512-1.

Supplementary Material 1.

Acknowledgements

Not applicable.

Author contributions

H.K. performed the experiments, analyzed the data, and wrote and edited the manuscript. Y.K. performed the experiments. H.C. and D.K. designed the study. T.K., as the corresponding author, supervised the study, provided support for data interpretation, and reviewed and revised the manuscript. All authors reviewed and approved the final manuscript.

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Data availability

The mass spectrometry-based proteomics dataset has been submitted to the ProteomeXchange Consortium through the PRIDE partner repository (http://www.ebi.ac.uk/pride/archive/) and is available under the dataset identifier PXD061076.

Declarations

Ethics approval and consent to participate

Written informed consent was obtained from all participants, and the study was conducted in full accordance with the protocol approved by the Institutional Review Board of Hanyang University (HYI-18-227-3). All procedures were performed in accordance with the relevant guidelines and regulations.

Consent for publication

Not applicable.

Competing interests

D.K. is an Editorial Board Member of Journal of Analytical Science and Technology. Editorial Board status has no bearing on editorial consideration.

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