SUMMARY

Gallbladder cancer (GBC) has a poor prognosis with the prevalence of GBC varying according to geographical location. The prevalence of GBC in South Africa is poorly tracked, and the molecular mechanisms associated with GBC in African patients are inadequately understood. This study aimed to determine dysregulated proteins in tissue and blood plasma in South African GBC patients to identify potential molecular mechanisms of disease progression and plausible biomarkers. Following ethical approval, tissue from 27 GBC, 13 gallstone disease (GD), and five normal tissues were obtained. Blood plasma was collected from 54 GBC and 73 benign biliary pathology (BBP) patients who consented to the study. A bottom-up proteomics approach was undertaken, using PAC and HILIC digestion methods, and SWATH-MS for quantitative proteomic profiling. Hierarchical cluster analysis, PCA analysis, and Spearman's rank correlation analysis were performed. Furthermore, pathway and network analyses were conducted. There were 62, 194, and 105 dysregulated proteins in the GBC/Normal, GBC/GD, and GD/Normal group comparisons, respectively, and 33 dysregulated proteins in the GBC/BBP plasma comparison. The dysregulated proteins in GBC patients enriched pathways involved in smooth muscle contraction, metabolism, extracellular matrix organisation and interactions, innate immunity, and platelet and neutrophil degranulation. Further analysis showed that S100A8 and S100A9 were downregulated in GBC plasma patients with GD history compared to those with no GD history. Additionally, APOE and ITIH3 were elevated in non-metastatic staging GBC patients. Seven proteins were found to be commonly dysregulated in GBC/GD and GBC/BBP comparisons and another two proteins were commonly dysregulated in the GBC/Normal, GBC/GD, and GBC/BBP comparisons, termed "Commonly dysregulated proteins (CDPs)". Quality control assessment of the MS2 fragment ion chromatograms of the CDPs indicated strong signal-to-noise ratios and correct fragment-to-precursor matching. The CDPs could distinguish between GBC and controls and the Spearman's rank correlation test showed significant correlations between the expression of the CDPs. The identified dysregulated proteins aid in further understanding the molecular mechanisms associated with GBC in patients with African ancestry. The alteration of specific proteins in tissue and plasma samples suggests their potential use as biomarkers for GBC patients in this sample cohort.