Chapter I

General Introduction

1.1 Gallbladder: Structure and function

The gallbladder (GB) is a small pear-shaped hollow organ, located beneath the liver. It is one of the principal organs of the biliary tract system (BTS). GB is located in the right upper quadrant of the abdomen and is affixed to the liver at the GB fossa. The cystic duct connects GB to the rest of the extrahepatic biliary system [1]. The gallbladder wall is composed of multiple layers. The innermost mucosal layer is composed of columnar epithelium with microvilli. Underneath the epithelium is an underlying lamina propria, a muscular layer, an outer perimuscular layer, and a serosa. The main function of the gallbladder is to store and concentrate bile, which allows the breakdown and absorption of dietary fats [2]. GB plays a vital role in the process of digestion. It facilitates the absorption of various substances such as Na^+, cholesterol, and proteins. Additionally, it aids in the accumulation of bile acids and the secretion of various substances, including glycoproteins, mucin, H^+ ions, Cl^-, immunoglobulins, and Ca^{2+}. The functions of GB are crucial for maintaining the overall health of the gastrointestinal tract. The intricate anatomical positioning of the GB [**Figure 1.1**] makes it susceptible to pathological complications. Dysfunction in GB physiology often gives rise to common issues such as cholelithiasis, gallstone (GS) formation, and the occurrence of gallbladder carcinogenesis [2-3].

1.2 Carcinoma of the gallbladder: Definition and pathophysiology

Gallbladder cancer (GBC) is the most frequent and aggressive cancer of BTS. GBC was first described by Stoll Maxmillian in 1777 and it was diagnosed incidentally during the histological examinations of the resected GB [4-5]. GBC is a highly fatal and multifactorial disease affecting mostly the elderly and female populations. It occurs twice in females as compared to males. Globally, GBC is a relatively rare malignancy but its incidence shows significant geographic variation, being particularly common in only certain regions or ethnicities. The complex disease biology, delayed presentation, and anatomical complexity make GBC a highly aggressive malignancy with an estimated five-year survival rate of only 5% [6–8]. The existence of various predisposing factors includes genetic inclination, geographical location, ethnicity, female gender, and chronic inflammation. The majority of the GBC cases are detected incidentally during cholecystectomy or at an advanced stage due to the aggressive nature of the tumor [9]. Surgical resection followed by chemotherapy is the only curative option for GBC detected at an early stage. Since most of the GBC patients present at an advanced or metastasized state, palliative care is the only treatment option available for GBC [10]. To date,

no targeted therapy is available for GBC patients. Hence, it is necessary to undertake immediate efforts to identify potential molecular signatures, enhancing the prognosis of GBC among vulnerable populations. Additionally, identifying GBC-specific cellular targets is crucial, laying the foundation for innovative therapeutic approaches.

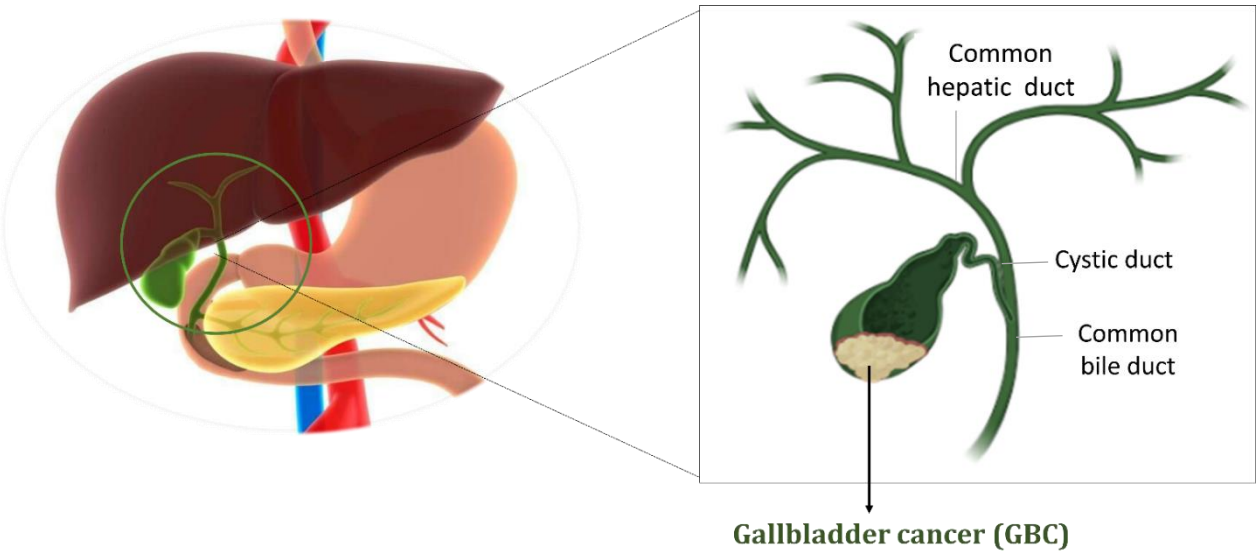

Figure 1.1: The anatomical position of the gallbladder in the hepatobiliary system.

1.3 Etiological factors associated with GBC

GBC is a multifactorial disease associated with numerous etiological factors. However, the underlying causes of GBC pathogenesis are poorly understood. GBC is associated with both environmental and genetic risk factors such as age, gender, ethnicity, geography, and family history which are unpreventable, and lifestyle factors, including dietary habits, obesity, and diabetes that can be prevented [11-12]. The burden of GBC is expected to rise further due to lifestyle and environmental factors [11]. The key etiological factors associated with GBC are cholelithiasis, female gender, bacterial infections, and environmental toxins.

1.3.1 Cholelithiasis and Cholecystitis

Cholelithiasis or gallstone disease (GSD) is one of the key etiological factors in GBC pathogenesis and is significantly linked to increased risk of GBC incidence and mortality rates. The large size, higher volume, and long duration of the gallstones have been reported to be the major risk factors associated with gallstones-related GBC cases [13]. Symptomatic GSD combined with acute cholecystitis or chronic inflammation is strongly involved in GBC development. Cholecystitis is caused by certain bacterial species associated with higher GBC

risk [14]. Bacterial infections caused by *Salmonella typhi* have about twelve times increased risk for gallstones development and GBC pathogenesis. In addition to *Salmonella spp*, *Helicobacter spp.* is associated with GBC pathogenesis. The distinct geographical variation of GBC incidence can be significantly correlated with salmonella infection and gallstone formation [15].

1.3.2 Gender disparity

The female gender is known to be one of the most important and independent risk factors associated with GBC. GBC demonstrates a prevalent gender bias worldwide, with a higher incidence in females as compared to males [6,16-17]. The female gender is at a higher risk for both GSD and GBC. Multiple case-control and cohort studies have demonstrated the association between female hormones and GBC risk. Moreover, geographic or ethnic variations in the GBC incidence are highly distinct in women, which indicates that there is presence of certain specific cofactors that might be associated with altered female hormone levels and contribute to GBC pathogenesis. Moreover, the incidence of GBC increases consistently with older age in females. It has been observed that two-thirds of GBC patients are over the age of 65 years [18-19].

1.3.3 Gallbladder pathologies

Pancreaticobiliary duct junction (PDJ) is an abnormal malformation of the BTS [6]. It is the premature union of the common bile duct and the pancreatic duct without the occupancy of the sphincter of Oddi. PDJ disgorges the pancreatic juices into the GB which leads to chronic inflammation of GB and bile stasis and finally results in malignant transformation of the GB cells [6,8,19]. GB polyps are defined as an abnormal growth or lesion that originates in the inner lining of the GB wall. The majority of the GB polyp cases are benign, but sessile polyps greater than 10 mm have an increased risk for GBC [14,20].

1.3.4 Environmental exposures

Several environmental factors including exposure to environmental toxins are involved in GBC development. GBC has been found in patients working in oil, paper, chemical, shoe, and textile manufacturing industries, indicating occupational exposure to chemical carcinogens. Several studies have also demonstrated the association of heavy metals with GBC. Other factors such as tobacco consumption, diet, family history, and obesity are involved with a higher risk of GBC development [21–23].

1.4 Clinical presentation and pathological features of GBC

1.4.1 Clinical manifestations

Due to the asymptomatic nature of GBC, the disease does not show any specific clinical symptoms in its early stages. However, as the cancer progresses, the GBC patients show nonspecific symptoms. The clinical manifestations of GBC have been categorized into the following five conditions:

(i) The first is acute inflammation. GBC patients with chronic inflammation tend to have an early malignancy stage and better survival outcomes.

(ii) Chronic infection falls under the second group.

(iii) Third syndrome is associated with biliary tract disease such as jaundice, weight loss, general weakness, and right upper quadrant pain. This clinical condition is associated with extensive disease in patients.

(iv) The clinical features of malignant tumors outside the biliary tract, which include anorexia, weight loss, general weakness, and local tumor complications such as a fistula or metastasis, fall into the fourth category. These patients typically have advanced disease.

(v) The final clinical symptoms include jaundice, benign manifestations outside the biliary tract, gastrointestinal bleeding, and upper gastrointestinal obstruction are common in this group of patients [19,23]..

In 15-20% of individuals, GBC is mostly detected incidentally during routine cholecystectomy or postoperatively by the pathologist. Whereas, about 80% of the GBC patients have locoregionally advanced disease with adjacent organ invasion or distant metastases [17].

1.4.2 Pathological features of GBC

Pathologically, GBC can develop in various types, but the most common histological subtype is adenocarcinoma which accounts for about 98% of all the GBC cases. The other histological subtypes include papillary, mucinous, squamous, and adeno squamous subtypes [24]. Two-thirds of the GBC cases are moderately or poorly differentiated adenocarcinoma. GB tumors may contain more than one histological subtype [25]. The disease stage determines the therapeutic options. Patients who qualify for cholecystectomy have improved survival outcomes. According to American Joint Committee on Cancer (AJCC) guidelines, GBC staging is done based on tumor invasion and the extent of metastatic spread [**Figure 1.2**]. In

stage I, the cancer cells infiltrate the lamina propria or the muscle layer. Perforation of serosa and/or involvement of adjacent organs or structures define Stage II. Tumor invasion from T1 to T3 with nodal involvement is automatically classified as stage II. Both stages I and II may be resectable with curative intent. Stage III disease is generally unresectable locally due to vascular invasion or the involvement of multiple adjacent organs. Stage IV denotes unresectability due to distant metastases [25-26].

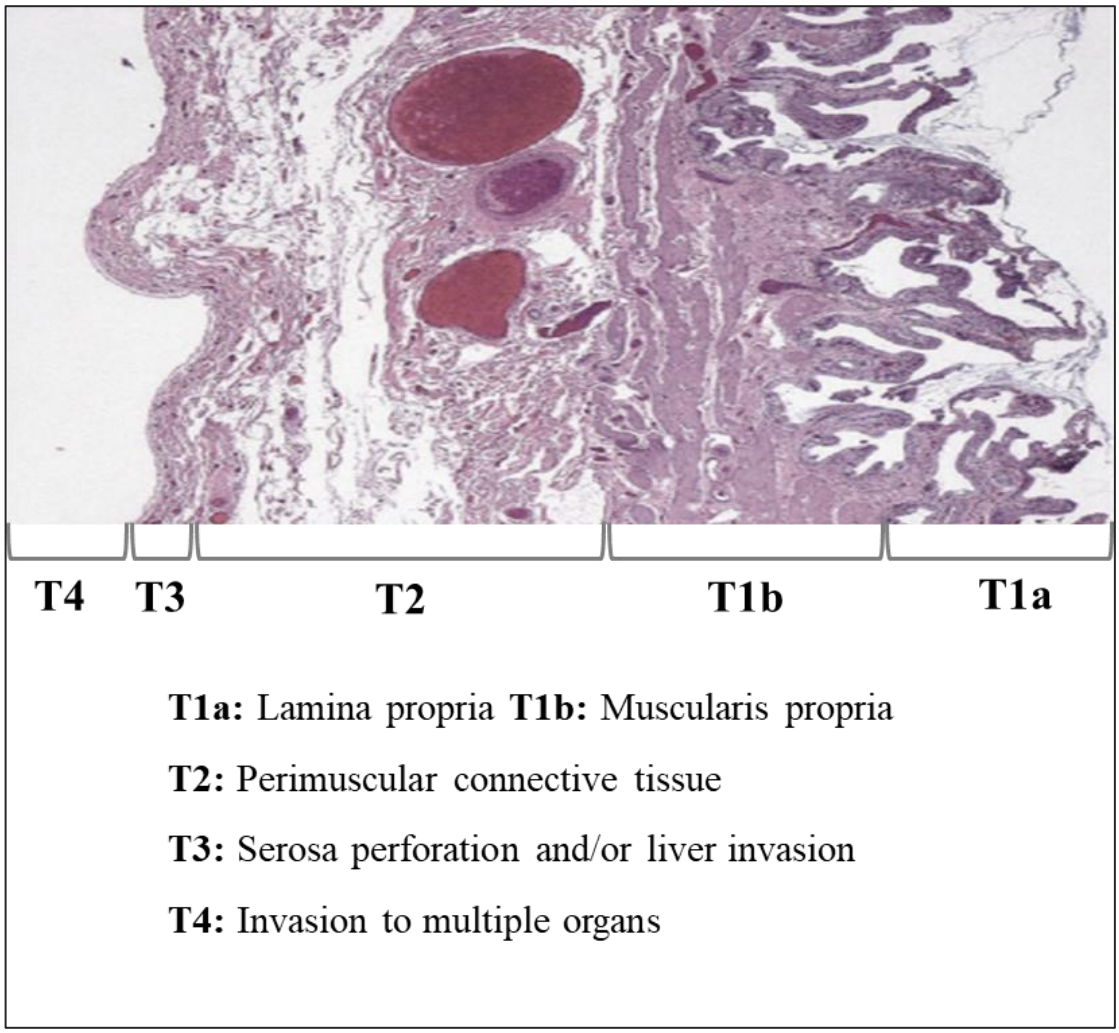

Figure 1.2: Histological representation of the gallbladder wall with the corresponding pathological stages of GBC (Reproduced from [14]).

1.5 Management of GBC

The management of this aggressive cancer is determined by several factors, including the stage of cancer, the position and size of the tumor, and the overall health of the patient. Surgical intervention is one of the primary treatments for GBC patients. Due to the aggressive nature of GBC, over half of the patients are not eligible for surgical resection and need to seek chemotherapy or adjuvant therapy [8]. Depending on the extent of tumor metastasis and resectability, operative resection is performed such as cholecystectomy wherein the GB is removed along with some part of the surrounding tissues [27]. In extremely rare cases of advanced GBC state, a surgical procedure, known as the "Whipple procedure" is performed which involves surgical removal of the portion of the small intestine and the pancreas along with the GB [28]. Currently, capecitabine, oxaliplatin, bevacizumab, and gemcitabine are being used for GBC treatment along with radiation therapy using fluorouracil. The National Comprehensive Cancer Network (NCCN) guidelines for GBC supports adjuvant fluoropyrimidine chemoradiation, fluoropyrimidine, or gemcitabine chemotherapy in patients with T1N0 GBC following curative surgery [29].

GBC is highly prevalent among the population of North and North-East (NE) regions particularly in women. GBC cases are addressed at metastasized or advanced stages and due to which, the therapeutic regimens often lack a significant response rate. According to the Global Cancer Observatory (GLOBOCAN) report published in 2012, the GBC burden in India especially, the NER will alone represent 10% of the world population by 2025. In contrast to other types of cancers, publicly available OMICs scale data on GBC is very rare. From this point, GBC can be seen as a neglected cancer type prevalent in NE India and Northern India. Comprehensive transcriptome-level molecular data on GBC and GSD patients from the worst affected NE Indian region is not available. Therefore, understanding the transcriptome architecture of GBC through systems-level approaches and unraveling the key molecular signatures and pathways associated with GBC pathogenesis are urgently needed.

1.6 Hypothesis

Based on the aforementioned rationale, the hypothesis posits the existence of distinctive and crucial molecular signatures in two specified categories of GBC: GBC associated with gallstones (GBC+GS) and GBC originating directly from a normal gallbladder without gallstones (GBC). Identifying and validating such unique molecular markers are anticipated to

be beneficial in precision medicine approaches, serving as biomarkers for GBC early detection, prognostic monitoring, and the formulation of new treatment strategies.

The research anticipated in this study aims to unravel the transcriptomic landscape to identify key pathogenic genes and pathways associated with GBC. Understanding the transcriptomic architecture of GBC and GSD and determining the noncoding and transcriptional regulatory networks in GBC would provide novel insights into GBC pathogenesis. The findings from this study would provide a better understanding of disease pathology and the unraveling of key molecular signatures and would help in facilitating further research toward the management of this aggressive disease.

Given these considerations, the thesis aims to address the following **objectives:**

1. Understanding the gallbladder cancer transcriptome and identifying potential genes, processes, and pathways associated with GBC pathogenesis.
2. Analysing noncoding RNA linked to GBC pathogenesis and constructing a ceRNA regulatory network to identify potential noncoding regulatory signatures in GBC.
3. Understanding the rewiring patterns of transcriptional regulation in GBC pathogenesis and identifying the crucial transcription factors involved in GBC development.
4. Validation of the identified key genes associated with GBC pathogenesis.

These objectives collectively constitute the comprehensive roadmap for exploring and identification of potential molecular signatures in GBC.

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