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Gallbladder Cancer- A Synoptic Review

Shahid Yousuf

School of Studies in Zoology,
Jiwaji University, Gwalior (Madhya Pradesh), India-474011

(Corresponding author: Shahid Yousuf, shahidyousufkmr@gmail.com*)*
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ABSTRACT: Gall bladder is the small sac-shaped organ beneath the liver, in which bile is stored after secretion by the liver and before releasing into the intestine. Gallbladder cancer involving the gastrointestinal tract is the fifth most common cancer occurring worldwide, but it is the most common malignant tumour of the biliary tract worldwide. Gallbladder cancer is now considered a distinct clinical entity, allowing for a separate analysis from that of other malignancies of the biliary tree. The marked improvement in the outcome of patients with gallbladder cancer in the last decade is because of the aggressive radical surgical approach that has been adopted, and improvements in surgical techniques and peri-operative care. To understand the mechanism and treatment of gall bladder cancer international collaboration is needed to enhance our knowledge. The present review encompasses the involvement of genetic mechanism of gallbladder cancer and focuses the elucidation of molecular mechanisms of gallbladder cancer. An effort towards the consolidation of these is made here.

Key words:

INTRODUCTION

The gallbladder is a small (about 3 to 4 inches long and normally not wider than 1 inch), pear-shaped organ under the right lobe of the liver, which concentrates stores and then re-secretes the bile, a fluid made in the liver. The gallbladder is divided into fundus, body, and the neck. The wall of gallbladder is composed of three layers mucosa, muscularis, and serosa. Bile helps digest the fats in foods as they pass through the small intestines, or is stored in the gallbladder and latter released. When foods (especially fatty foods) are being digested, the gallbladder contracts and releases bile through a small tube called the cystic duct. The cystic duct joints up with hepatic duct, which comes from the liver, to form the common bile duct. The common bile duct joins with the main duct from the pancreas (the pancreatic duct) to form the ampulla of vater which then empties the bile into the duodenum. The ampullary opening into the duodenum is controlled through the muscular sphincter of oddi (Bartlett *et al*., 1994; Wistuba and Gazdar, 2004).

Gallbladder cancer

Gallbladder cancer (GBC) is the most frequent malignant tumor of the biliary tract and the fifth most common cancer of the digestive tract. Approximately 60 percent of tumors originate in the fundus of the gallbladder, 30 percent originate in the body, and 10 percent originate in the neck (Albores-Saavedra *et al*., 1986). A review of the pathology of gallbladder malignancy reveals that gross descriptions of GBC can be grouped into infiltrative, papillary, nodular and combined forms. Infiltrative forms present with gallbladder wall thickening, papillary forms with polypoidal lesions with frond like projections and nodular forms present with more circumscribed masses. Most tumors, however, have an infiltrative pattern as at least a part of their presentation, causing

thickening and in duration of the gallbladder wall (Sumiyoshi *et al*., 1991).

Histologically, the most common type of GBC is adenocarcinomas (80-95%). Less common types of GBC are undifferentiated or anaplastic carcinoma (2–7%), squamous cell carcinoma (1–6%), and adenosquamous carcinoma (1–4%) and other types, such as adenosquamous carcinoma, oat cell carcinoma and sarcomas, have also been described. Rare primary histologies such as carcinoid, lymphoma, and melanoma have been reported. Adenocarcinomas can be divided into multiple subtypes, including well-differentiated, papillary, intestinal type, pleomorphic giant cell, poorly differentiated small cell, signet ring cell, clear cell, colloid and the choriocarcinoma-like cell subtype. The papillary histological subtype has the best prognosis whereas the poorly differentiated small cell tumor has the worst prognosis (Albores-Saavedra *et al*., 1986). The majority of cases are diagnosed in the advanced stages, leading to extremely poor prognosis. The prognosis is mainly dependent on histological subtype, grade, and stage of the tumor at the time of presentation. The overall mean survival rate for patients with GBC is 6 months, with a 5-year survival rate of 5% (Levy *et al*., 2001).

The prevalence of GBC shows great geographical variation. Though an uncommon malignancy, it is the fifth commonest gastrointestinal malignancy (following colon, pancreas, stomach and esophagus) and the most common biliary tract malignancy in the USA. It is rare in the western world, including the USA, UK, Canada, Australia and New Zealand, where the incidence rates range between 0.4 and 0.8 in men and between 0.6 and 1.4 in women per 100,000 populations. However, high incidence rates, up to 2–4 in men and up to 4–6 in women, have been reported from various countries in central and south America, central and eastern Europe, and Japan. In Chile, GBC is the leading cause of death from cancer among women (Kapoor and McMichael, 2003).

Various epidemiological reviews have reported that GBC is rare in India. These observations are based on incidence rates of 0.5 and 1.3 per 100,000 populations in men and women, respectively, reported from Mumbai in western India. However, the incidence of GBC varies widely within India. GBC is much more common, especially in women, in north and central India than in the west and south. Though the overall age-adjusted incidence rates of GBC in India are low (1.0 for men and 2.3 for women per 100,000 population), the incidence in women in Delhi in north India and Bhopal in central India is as high as 6.6 and 5.2, respectively, much higher than 0.6 in Chennai and 0.8 in Bangalore in south India.

GBC increases considerably with age with a mean age of the patients, 65 years and the highest incidence of the disease occurs in the seventh and eighth decades of life (Piehler and Crichlow, 1978). Women are 2-6 times more likely to develop GBC than men in United States. The large geographical differences in the incidences of GBC suggests the presence of nutritional and environmental factors in the etiology of the malignancy, in additional to genetic, ethnic, racial and social factors (Diehl *et al*., 1983). Several factors have been associated with the risk of developing GBC, Lithiasis, being one of the main risk factors, presenting in 65% to 90% of cases of GBC (Lazcano-Ponce *et al*., 2001). The risk is also associated with the number and size of the stones (Chow *et al*., 1999). Likewise, and closely connected with lithiasis, chronic gallbladder inflammation might induce the continuous release of inflammatory mediators and growth factors (tumor promoters), which exert their effect on an epithelium previously damaged by carcinogenic agents (Macarthur *et al*., 2004). The other risk factors for developing GBC include chronic infection of the biliary tract, in particular due to *Salmonella typhii*, abnormal junction of the pancreatic and biliary duct, porcelain gallbladder, gallbladder polyps, chemical exposure, cigarette smoking, high parity, post-menopausal state, a high fat and carbohydrate diet, obesity, multiple pregnancies, and the use of estrogens (Calle *et al*., 2003; Pandey *et al*., 2003).

The symptoms of GBC are nonspecific, but the risk is significantly higher in cholelithiasis. The non-specificity of symptoms is responsible for delayed diagnosis; in fact, this tumor is usually identified at an advanced stage when it has already become unresectable. The prognosis of GBC is poor and less than 5% of the patients remain alive, five years post operation. The major route of spread of GBC is loco regional; in fact, it usually extends directly into the liver and portahepatis resulting in narrowing or obstruction of the common hepatic or right hepatic duct (Artico *et al*., 2010). The patients of the gallbladder most often become symptomatic when the cancer obstructs the drainage of the bile; the bilirubin pigment of bile accumulates in the blood, causing jaundice. The jaundice is usually associated with itching of the skin (also called, pruritus). The body compensates partially and excretes some of this bilirubin via the urine, so patients may have dark (cola colored) urine. Because bile cannot reach

the intestine, the patient's stool become white (clay colored). Patients with GBC may have pain in the right upper portion of the abdomen. This pain is a result of inflammation of the gallbladder due to the blockage of the cystic duct. Besides these, the other symptoms are fever, anorexia, nausea, vomiting and body weight loss. (Cancer Research UK, 2012)

Gene mutations related to GBC are usually acquired during life rather than being inherited. Among the most common molecular changes present in tumors, including GBC are mutations of the *TP53* tumor-suppressor gene, activating mutations of the *KRAS* proto-oncogene and loss of cell-cycle regulation. Other genes that may play a role in GBC include *BRAF*, *FHIT*, *CDKN2*, and *HER2* (Wistuba and Albores-Saavedra, 1999). Besides the multistep genetic alterations that lead to tumor genesis in gallbladder and gall stone diseases, the sequential epigenetic processes have also been linked to GBC formation; one of these epigenetic alternations is DNA methylation which is associated with loss of gene expression in solid tumors (Bird AP, 1986). The most commonly methylated genes in the GBC were *p16* (56%), *p73* (28%), *APC* (27%), *hMLH1* (14%), *SEMA3B* (92%), *FHIT* (66%), *BLU* (26%) *DUTT1* (22%) and *RASSF1A* (8%).

Telomeres are heterochromatic structure at the ends of the chromosomes formed by tandem repeats of TTAGGG sequences and bound by array of specialized proteins that form a protective structure known as shelterin (de Lange, 2005). The shelterin complex helps to maintain telomere integrity by protecting the telomeres from chromosomal abnormalities and DNA-damage responses due to telomere replication, recombination and erosion and serves to protect chromosomes ends. DNA damage responses due to telomere dysfunction and in presences of telomere maintenance mechanism: telomerase or alternative lengthening of telomeres (ALT) cells, may bypass crisis and become immortalized leading to carcinogenesis by promoting genetic instability (Garcia-Aranda *et al*., 2006).

Telomeric DNA repeats cannot be methylated however adjacent subtelomeric DNA is heavily methylated in humans. In a study of telomerase negative cells subtelomere chromatin changed in to an open structure upon telomere shortening (Benetti *et al*., 2007). Recent studies have shown that de-methylation of subtelomeric regions in DNMT-deficient cells results in telomere lengthening caused by increased homologous recombination in telomeric sequences.

Hence, the present study aims at studying the methylation pattern of subtelomeric sequences and to see if it by any means correlates with the different grades of GBC or gall stones. The gallbladder is a small, hollow, pear-shaped pouch in the body. It lies underneath the right side of the liver, in upper abdomen. It is about 7-10 cm long and 3-4 cm in width. The gallbladder is a hollow organ that concentrates and stores bile. It lies in the gallbladder fossa on the inferior aspect of the right lobe. It has a rounded fundus, a body, and an infundibulum and its wall is composed of three layers, mucosa, muscularis and serosa. Gall stones may become impacted in small bulge in infundibulum known as Hartmann's pouch (Frierson H, 1997).

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The cystic duct from the gallbladder joins the common hepatic duct to form the common bile duct, usually about 5 cm above the duodenum. Rarely, an accessory cystic duct (duct of Luschka) drains bile intra-hepatically through the gallbladder fossa, and is susceptible to injury during cholecystectomy. The left and right hepatic ducts unite at the base of segment, anterior to the portal vein bifurcation. The common hepatic duct passes inferiorly in the right edge of the hepato-duodenal ligament, to the right of the common hepatic artery, and joins the cystic duct to become the common bile duct. The common bile duct (diameter 3–7 mm) passes behind the first part of the duodenum, enters the head of the pancreas, and terminates at the Ampulla of Vater (Laurh *et al*., 2001). The blood supply of the biliary tree is derived from the hepatic artery, which explains the presence of biliary complications that develop after hepatic artery thrombosis in liver transplant recipients (Laitio, 1980). Calot's triangle is bordered by the gallbladder, the common hepatic duct and the liver. The peritoneal covering of the gallbladder extends onto the anterior and posterior aspects of Calot's triangle and onto the portal structures. The arterial supply of the gallbladder is via the cystic artery, which usually arises from the right hepatic artery and lies within Calot's triangle.

Occasionally, the cystic artery has anterior and posterior branches before entering the gallbladder (Laitio and Nevalainen, 1975). Like all other parts of the body, the area containing the gallbladder also contains lymph nodes. These nodes are called lymph glands. They are small bean shaped glands that are part of the lymphatic system.

Functions of Gallbladder

The main functions of the gallbladder are storing bile produced by the liver and modifying its composition before releasing it into the duodenum. Bile filling of the gallbladder is promoted by contraction of the sphincter of Oddi. Hepatic bile, consisting of an isotonic fluid with an electrolyte composition resembling blood plasma, is concentrated within the gallbladder by transmucosal absorption of water and electrolytes. The concentrated bile secreted into the duodenum consists of micellar, globular lipid molecules suspended in water, with the non-polar ends of these molecules facing inwards and their polar tails facing outwards. This suspension facilitates the intestinal absorption of dietary lipids across the absorptive cells of the brush-border membrane of the intestine, either through passive diffusion or by a carrier-mediated mechanism. The main factor controlling the evacuation of the gallbladder contents into the duodenum through contraction of the muscular layer is the peptide hormone cholecystokinin, which is released from the duodenal mucosa in response to the ingestion of fats and amino acids (Wistuba and Gazdar, 2004). Biliary tract carcinomas(BTCs), which include cancers of gallbladder and intra and extra hepatic biliary tree, consisting of various ducts that carry bile, are relatively infrequent, but highly lethal diseases that are notoriously dificult to diagnose and treat (Wistuba and Gazdar, 2004).

Epidemiology

GBC is a rare neoplasm with varying demographic distribution in different parts of the world. Though this type of cancer is uncommon in U.S and Europe, it is more common in Chile, Peru, Japan and Korea (Curado *et al.,* 2007). GBC is an infrequent neoplasm in most western countries but is most common in some other parts of the world. The highest GBC incidences have been reported in women from India (21.5/100,000), Chile (18.1/100,000), Pakistan (13.8/100,000) and Ecuador (12.9/100,000). High incidences have also been found in Korea, Japan and in some central and eastern European countries such as Poland, the Czech Republic and Slovakia (Randi *et al.,* 2006). GBC is up to three times higher among women than men in almost all populations but vary from 1 in Far East Asia to 5 in Spain and Columbia (Roa *et al.,* 1994, Randi *et al.,* 2006). In India, GBC shows varying geographic distribution, as the incidence is much higher in urban Delhi population compared to south India national. The reported incidence ranged from 10/100,000 in Delhi to 2-3/100,000 in South India (Pandey *et al*., 2008). It is much higher in northern cities (e.g. incidence in Delhi is 7/100,000 for male and 8.9/100,000 for female and in Bhopal it is 1.6 and 2.5 per 100,000 for male and female respectively) as compared to southern cities (e.g. in Chennai incidences is 0.5/100,000 for male and 0.8/100,000 for female and in Bangalore incidence for male is 0.6/100,000 and for female it is 0.7/100,000 population. The incidence rate reported in Indian Council of Medical Research cancer registry from the other incidence districts/cities of the India like Kamrup (8.1), Kolkata (5.4) and Mumbai (3.2) were less than in north central India region (National Cancer Registry Aug 2001).

This larger geographic difference in the incidence of carcinoma of the gallbladder suggests the presence of nutritional and environmental factors in the etiology of the malignancy in addition to genetic, ethical and social factors (Diehl *et al.,* 1983) and so far the main associated risk factors identified includes cholelithiasis (especially untreated chronic symptomatic gall stones) obesity, reproductive factors, chronic infection of gallbladder and environmental exposure to specific chemicals GBC increases considerably with age. The mean age of patients with carcinoma of the gallbladder is 65 years and the highest incidence of the disease occurs in the seventh and eighth decades of life (Piehler and Crichlow, 1978).

Risk factors

GBC is the most common malignant tumor of the biliary tract and is fifth most mortality causing cancer. It is one of the obesity associated cancers and positively correlates with prolonged cholelithiasis (gall stone) and cholecystitis. Besides these other risk factors include gallbladder polyps, anomalous pancreaticobiliary duct junction, chemical carcinogens, and chronic infections.

Cholelithiasis (gall stones)

Gall stones are the major risk factors for developing GBC and are present in between 60-90% of cases in different populations around the world (Roa *et al.,* 1994; Hsing *et al.,* 2007). Gall stones are hard, rock like formation of cholesterol and other substances that form in gallbladder and

cause chronic inflammation. A cholesterol gall stone represents approximately 80-90% of all gall stones cases in the western world and is considered to be promoting factor (Lazcano *et al.,* 2001). It has been recently reported that the increase in the size of gall stones could be related to greater risk of GBC (Vitetta *et al.,* 2000). This is exemplified by the relative risk of GBC and with gall stones size, if the stones are 2.0-2.9 cm in diameter then relative risk was 2.4 and increases up to 3 if size of stone was 3.0 cm (Towfigh *et al.,* 2001).

However the increase in number and size of the stones among the patients with GBC could simply be an effect of aging or a reflection of the long term presence of stones in the gallbladder and the underlying genetic or lifestyles determinants of stones within families contribute the risk of biliary tract cancer outside the gallbladder (Hsing *et al.,* 2007).

Porcelain Gall bladder

Porcelain gallbladder is a condition in which wall of the gallbladder becomes covered with calcium deposits. A strong association has been reported between porcelain gallbladder and GBC (German *et al.,* 1979). The pathological findings of brittle gallbladder with bluish discoloration resulting from extensive calcification of the organ wall, has been associated with carcinoma in 12.5-62% of patients suffering from GBC (Towfigh *et al.,* 2001).

Anomalous Pancreatobiliary Ductal Junction

Anomalous Pancreatobiliary Ductal Junction (APDJ) is a rare congenital anomaly considered to be an etiological factor in the development of carcinoma of the biliary tract (Sasatomi *et al*., 2000) especially in the relatively young female patients with no gallbladder stones (Kang *et al.,* 2007). The APBDJ specifically related to papillary carcinoma of gallbladder is rare in western countries than in Japan and is less invasive and fatal than other carcinomas of gallbladder (Nuzzo *et al.,* 2005). The APBDJ between the common bile duct and pancreatic duct is not under the control of sphincter and the premature junction results in regurgitation of pancreatic juice into the gallbladder. Refluxing pancreatic juice results bile changes and induces chronic inflammation and increased cell proliferation, leading to epithelial hyperplasia, metaplasia and carcinoma of biliary tract (Chao *et al.,* 1999).

Chronic Inflammation and Infection

The most causative factor for GBC is chronic inflammation of gallbladder wall/mucosa and leads to mucosal dysplasia and subsequent carcinoma (Albores-Saavedra *et al.,* 1986). The cause of gallbladder mucosal inflammation include infection drugs (Isoniazid and methyl dopa), and congenital anomalies (Bartlett *et al.,* 2000). The causative factors leads to chronic inflammation and production of certain toxins and metabolites with carcinogenic potential all involved in transformation of gallbladder epithelium. Certain bacteria like *Salmonella typhii*, *Helicobacter bilis*, *H. hepaticus and E. coli* have been mediators of chronic inflammation and have been implicated into carcinogenesis (Nath *et al.,* 2010), and besides this the patient with cholelithiasis develop chronic inflammation of the gallbladder and finally leads to GBC (Lowenfels *et al.,* 1985).

Pollutants and Environmental Factors in GBC

A recent meta-analysis has revived these factors (Pandey *et al.,* 2006). The number of heavy metals like Cadmium, Nickel and Zinc has been implicated but the evidence is not sufficient to confirm an association. Besides these, other chemical agents that effect in experimental animals like Methylcholanthrene, o-aminoazotoluene and nitrosamine cause GBC (Albores-Saavedra *et al.,* 1986). The occupational exposure in rubber industry and in North India the use of mustard oil loaded with carcinogenic impurities has been suggested as a risk factor (Hai *et al.,* 1994).

Dietary Factors and Obesity

The epidemiological studies worldwide have implicated dietary factors in the development of GBC. An increased risk has been observed with obesity, high intake of calories, high carbohydrate and greater preference for oily foods (Zatonski *et al.,* 1997). A risk of GBC was associated with high consumption of red chili pepper (Pandey *et al.,* 2006). Vegetables and fruits have protective effect and there has been some suggestion of inverse association with fiber intake, Vitamin C and Vitamin E (Arundhati *et al.,* 2004). The monosaccharides and disaccharides are potential risk factors of GBC. Sugars may influence the bile composition through lipoprotein metabolism (Moermann *et al.,* 1995). Obesity is associated with increased risk of GBC like in other cancers. In over 84,000 men and 97,000 women included in the Cancer Prevention Study II Nutrition Cohort, the relative risk of GBC was 1.8 (95% confidence interval [CI], 1.1 to 2.9), in obese men with a BMI of 30.0 to 34.9 compared to men with a normal BMI (18.5 to 24.9). Obese women (BMI, 30.0 to 34.9) had a relative risk of 2.1 (95% CI, 1.6 to 2.9) compared to women with a normal BMI (Larrson *et al.,* 2007).

Gallbladder Polyps

The presence of adenomatous polyps within the gallbladder can be risk factor for carcinoma. Sessile or pedunculated adenomatous polyps can be precancerous or can harbor occult invasive malignant disease. The risk of occult malignancy correlates with polyp size (Zielenski *et al.,* 2009). The polyps larger than 10mm in diameter have greatest malignant potential and small polyps less than 10mm in diameter need only be removed if they are producing symptoms or associated with gall stones (Aldridge *et al.,* 1990).

Diagnostic Features, Histology and Staging in GBC

The various symptoms associated with primary GBC make the early diagnosis of this uncommon entity a challenging task. The non-specific symptoms have been grouped into five clinical syndromes (Pichler *et al.,* 1978). *Acute cholecystitis*: - About 1% of patients for acute cholecystitis has an earlier stage of carcinoma and has improved survival and the second group is *chronic cholecystitis*. The third syndrome is *biliary tract diseases* with symptoms jaundice, weight loss, general weakness, pain in right upper quadrant. The fourth category refers to *malignant tumors* outside the biliary tract, with symptoms of weight loss, general weakness and anorexia and local complications of tumor and the last category is *benign manifestations* outside the biliary tract. The small group of patients with this syndrome has gastrointestinal bleeding and upper gastrointestinal obstruction.

Adenocarcinoma is the most frequent histological type found in GBC. It represents 80% to 95% of all tumors, and the most frequent forms are moderately or poorly differentiated (Misra *et al., 2003)*. Two carcinogenic models of GBC sequence are recognized: the metaplasia-dysplasia carcinoma and the adenoma-carcinoma, which have origins in two different types of epithelial lesion in the gallbladder. The metaplasia-dysplasia-carcinoma sequence, the most significant and frequent type of gallbladder carcinogenesis, is based on alterations to the epithelium of the gallbladder mucosa. The metaplasia frequently appears as an adaptive process secondary to chronic irritation or inflammation. Dysplasia appears on top of this metaplasia, which progresses to carcinoma in situ and subsequently becomes invasive (Figure 2). Severe dysplasia and carcinoma in situ have been found in more than 90% of GBC (Gourgiotis *et al.,* 2008). Less frequent is the second pathway (adenomacarcinoma sequence), which suggests a malignant transformation from an adenomatous lesion (Aldridge *et al*., 1993).

The metaplasia frequently appears as an adaptive process to chronic inflammation generally produced by a gall stone. Dysplasia appears on top of this metaplasia, which progresses to a carcinoma in situ and subsequently becomes invasive (Letelier *et al*., 2008).

The TNM staging system of the International Union Against Cancer (UICC) and American Joint Committee on Cancer (AJCC), which has proven to be a good system for comparison of surgical results and prediction of patient outcome. Briefly, under the TNM classification: Stage I is a tumor limited to mucosa or muscular layers; Stage II tumors invade the peri-muscular tissue; Stage III tumors invade serosa, liver less than two centimeters, or have regional (hepato-duodenal ligament) lymph node metastasis; Stage IV shows liver invasion greater than two centimeters (Stage IVA), or metastasis to non regional lymph nodes and/or distant organs (Stage IVB).

Table 1: Tsukada *et al* reported the five year survival rate in patients with TNM stages.

Stage	Survival Rate
I	91%
II	85%
III	40%
IV	19%

Molecular and Genetic Alterations in GBC

Existing information with respect to the alterations observed in GBC at a genetic and molecular level is still limited. As with other neoplasms, GBC is a product of the accumulation of multiple genetic alterations. Initial results show the participation of oncogenes, tumor suppressor genes and DNA repair genes as well as microsatellite instability and important epigenetic alterations represented mainly by methylation of the gene promoter areas (Lazcano *et al.,* 2001). Until now, it has not been possible to establish with clarity a sequence of events leading to gallbladder carcinogenesis.

Loss of Heterozygosity

The most representative complex mechanism responsible for the inactivation of tumor suppressor genes involves the mutational events of one allele and allelic loss of the other allele (Kuroki *et al.,* 2005). The allelic loss can be detected as loss of heterozygosity (LOH) by using microsatellite markers. On chromosomal arm 1p, 3p, 5q, 8p, 9p, 9q, 13q, 16q and 17q in GBC (Kuroki *et al.,* 2005), while loss of heterozygosity (LOH) on 13q and 18q is frequent in higher grades

(III and IV) of GBC (Chang *et al.,* 1999) and in dysplasia loss of heterozygosity on 3p, 5q, 9p, 13q, 16q and 17q indicates early changes in GBC pathogenesis (Table 1) (Chang *et al.,* 1999; Kuroki *et al.,* 2005).

Table 2: Loss of Heterozygosity in Gallbladder Cancer.

Chromosome Region	Incidence %	Associated gene
1p 34-36	53	P73
3p	76-100	VHL, RAR-3, RASSFIA, FHIT
5p21	66	APC
8p21-23	100	PRLTS, FEZI
9p21	38-60	P15, P16
13q14	20-56	RB
16q29	61	WWOX
17p13	42-91	P53

Dominant Proto-Oncogene

The *ras* gene family (N-ras, H-ras, K-ras) code for homologous protein of 21kD, which is involved in signal transduction pathway of the cell cycle. K-ras point mutation affects codons 12, 13 and 61 resulting in continuous and inappropriate growth signals (Boss *et al.,* 1988). A great frequency of K-ras mutation (50-80%) has been reported in GBC patients with APBDJ, indicating the reflux of pancreatic juice has a role of occurrence of these mutations (Hanada *et al.,* 1999). The erb-B2 proto-oncogene encodes a transmembrane receptor tyrosine kinase that has an important role in co-regulation of DNA repair, cell cycle check points and apoptosis. ERBB2 over expression has been detected in 33-64% of GBC (Kim *et al.,* 2001).

TP53 Alteration (Tumor Suppressor Genes)

The TP53 tumor suppressor gene, located on short arm chromosome 17 (17P) encodes 53kD nuclear transcription factor particular in response to DNA damage by γ-radiation, UV- radiation and carcinogens (Greenblatt *et al.,* 1994), and plays major role in maintaining the integrity of the genome since loss of p53 function allows inappropriate survival of genetically damaged cells and leads to cancer (Levin, 1997). TP53 are frequent in GBC and there is no apparent geographic variation in the incidence of these mutations (Hanada *et al.,* 1995).

CDKN2A-p16

CDKN2A-p16 is a tumor suppressor gene that encodes the protein p16, which is an inhibitor of cyclin dependent kinase, involved in cell cycle regulation at checkpoint G1. The loss of p16 expression is usually connected to homozygote deletion, loss of heterozygosity, mutations and methylation. Inactivation of p16 through methylation of the promoter region has been frequently identified in breast, prostate, head and neck, liver, lung, brain, colon and esophageal cancers and cell lines of bladder cancer (Liggett *et al.,* 1995). This tendency is also observed in GBC with a loss of expression of up to 62.5% (Tadokoro et a., 2007). Other investigations have identified a methylation percentage of 72.5% at different stages of progression; nevertheless, a significant relationship to the loss of expression of this protein was not established (Tadokoro *et al.,* 2007). The methylation state of this gene was evaluated in samples from the US and Chile, methylation frequencies of 56% was recorded, with similar methylation patterns in both populations (House *et al.,* 2003).

UCHL1

UCHL1 (also known as PGP9.5) is the only gene with a potential oncogenic role that has found to be hypomethylated in the promoter region in GBC (Lee *et al.,* 2006). It is located on chromosome 4p14 and was identified originally as a member of a gene family whose products hydrolyze small C-terminal adducts of ubiquitin (Ub) to generate the ubiquitin monomer (Liu *et al.,* 2002). The product of the gene is a peptide responsible for eliminating Ub from proteins that have it, and to thereby avoid its degradation by the proteosome. Proteins degraded by this mechanism actively participate in cell cycle control, for example, p53 and a variety of cyclins (Ishibashi *et al.,* 1991). In GBC, a progressive decrease in the methylation of this gene has been observed, with 84.6% in normal epithelium, 37.5% in adenoma and 27.2% in carcinoma. These results suggest that hypomethylation of the PGP9.5 promoter is a reliable marker in GBC and that DNA hypomethylation might play a significant role in the re-expression of the gene in GBC (Lee *et al*., 2006).

Overview of epigenetic mechanisms

The current field of epigenetics includes a number of mechanisms, including DNA methylation,

histone modification, and microRNAs (Chuang *et al.,* 2007; Allis *et al.,* 2007). DNA methylation is a covalent modification, heritable by somatic cells after cell division. 5-methyl-cytosine (5MeC) represents 2-5% of all cytosines in mammalian genomes and is found primarily on CpG dinucleotides (Millar *et al.,* 2003). DNA methylation is involved in regulating many cellular processes, including chromatin structure and remodeling, X-chromosome inactivation, genomic imprinting, chromosome stability, and gene transcription (Grewal *et al*., 2003; Reik *et al.,* 2001). Generally, gene promoter hypermethylation is associated with decreased expression of the gene (Orphanides *et al*., 2002).

Histones are globular proteins that undergo post-translational modifications that alter their interaction with the DNA and other nuclear proteins (Kouzarides *et al*., 2007). H3 and H4 histones have long tails protruding from the nucleosome, which can be covalently modified by acetylation, methylation, ubiquitination, phosphorylation, sumoylation, citrullination, and ADP ribosylation, and thus influence chromatin structure and gene expression.

MicroRNAs (miRNA) are single-stranded RNAs of 21–23 nucleotides in length that are transcribed from DNA but not translated into proteins (non-coding RNAs); mature miRNAs are partially complementary to one or more messenger RNA (mRNA) molecules. miRNA main function is to down-regulate gene expression by interfering with mRNA functions (Jackson *et al*., 2007; Pillai *et al*., 2007).

Epigenetic Alteration in cancer

Besides the multistep genetic alterations that lead to tumorgenesis in variety of human tissues, the sequential epigenetic processes have also been linked to human cancers formation. One of these epigenetic alternations is DNA methylation which is associated with loss of gene expression in solid tumors (Bird AP, 2001). The first alteration is loss of methylation in normally methylated CpG sites (in repetitive and/or endoparasitic sequences) and is known as overall genomic hypomethylation. The second alteration, characterized by an increase of methylation in CpG islets in normally demethylated regulatory regions, is known as aberrant hypermethylation. Many tumor suppressor genes are silenced by DNA methylation during carcinogenesis (Esteller *et al.,* 2002). Typically, dense regions of CpG dinucleotides, termed CpG islands, within tumor suppressor gene promoters are protected from methylation in normal mammalian cells, and transcription is unaffected. During carcinogenesis, however, aberrant promoter-region methylation accumulates in tumor-suppressor genes, resulting in blocked transcription (Baylin, 2001).

Aberrant Methylation in Preneoplastic Gallbladder Lesions

In a study by House *et al.,* 2003), the hypermethylated state of six tumor-associated genes in a normal gallbladder, chronic cholecystitis and adenocarcinomas (samples fixed in formalin and embedded in paraffin) in a series of Chilean patients was conducted. The patients with chronic cholecystitis showed 28% methylation in some of the genes (APC, p16 and hMLH1). Likewise, the gene methylation pattern in preneoplastic and neoplastic gallbladder lesions of these genes DAPK-1, DLC-1, TIMP-3 and RARβ-2 presented a progressive increase in their state of methylation from chronic cholecystitis to advanced carcinomas and at the same time an aberrant methylation pattern of the gene for E-cadherin (CDH1) with a progressive increase in the methylation from chronic cholecystitis without metaplasia to advanced carcinoma (53% to 65.2%) was shown (Garcia *et al.,* 2009).

Aberrant Methylation in Gallbladder Cancer

The published studies have made it possible to establish that transcriptional gene silencing is due to the methylation state of its promoter regions, a mechanism that is alternative to mutation and allelic deletions. This seems to be an early, progressive and cumulative event in GBC, which increases from chronic cholecystitis without metaplasia to metaplasia. The variation of methylation frequencies in cases of different geographical origin, suggests population differences, is worthy of note, because similar results have been observed in a study of genetic alterations (mainly mutations) (House *et al.,* 2003). For example, in Chilean patients, it has been reported SHP1 (80%), 3-OST-2 (72%), CDH13 (44%), P15INK4B (44%), CDH1 (38%), RUNX3 (32%), APC (30%), RIZ1 (26%), P16INK4A (24%) and HPP1 (20%) presented a high percentage of methylation in patients with GBC (Table 2) (Takahashi *et al.,* 2004). The methylation state in CDH13 (69.6%), DAPK1 (60.9%), FHIT (56.5%) and RAR beta 2 (43.5%), genes which presented a high methylation frequency in advanced GBC in Chilean patients showed in Table 2 (García *et al.,* 2009). In addition, both of them found that the methylation state of DLC1 was an indicator of poor prognosis, and methylation of MGMT is correlated with better survival. Other authors evaluated the methylation states of APC and FHIT and their

relationship to survival, with methylation percentages of 40% and 30%, respectively. Epigenetic inactivation by methylation in chromosome 3p is a frequent event in patients with GBC, particularly affecting the promoter region of the tumor suppressor genes SEMA3B (3p21.3) and FHIT (3p14.2) with 92% and 66% methylation, respectively (Riquelme *et al.,* 2007). It was found that the methylation in exon 1 of ASSF1A gene was 36.4% in carcinoma samples, 25.0% in adenoma and 8.0% in normal epithelium of gallbladder (Kee *et al.,* 2007; Tozawa *et al.,* 2004).

Alterations in DNA methylation patterns are commonly found in all cancers, often with concomitant changes in gene expression. In GBC, molecular information is reduced; however, a high rate of methylation of some genes in GBC has been reported and associated with carcinogenesis of other tissues of the human digestive tract. The acquisition of hypermethylation at multiple tumor-suppressor gene-promoter sites may contribute to tumor formation and progression within the chronically inflamed gallbladder. The most commonly methylated genes in the GBC were *p16* (56%), *p73* (28%), *APC* (27%), and *hMLH1* (14%). Significant differences in gene methylation were discovered between US gallbladder cancers and those from Chile, where gallbladder cancer is one of the leading causes of cancer-related deaths. *APC* methylation was present in 42% of the US cases but in only 14% of the Chilean tumors *P73* methylation was common among the Chilean cancers (40%) compared with those from the United States (13%) (Michael *et al.,* 2003).

Table 3: Summary of the methylation rate of multiple genes studied in advanced GBC (Letelier *et al*., 2012).

Gene name	Full name	Function	Frequency of methylation % (n)	Origin of specimen
CDH1	Cadherin 1, type1, E cadherin (epithelial)	Tissue invasion (cell-cell adhesion)	11 (1/9) 38 (19/50) 65 (13/20) 60 (13/20) 65 (15/23) 41 (9/22)	Japan Chile Chile Chile Chile Japan
APC	Adenomatous polyposis coli	Cell migration, adhesion and apoptosis	26 (14/54)	Chile, USA
hMLH1	Human homologs of the MutL gene of bacteria	Mismatch repair	13 (7/54)	Chile, USA
p16	Cyclin-dependent kinase inhibitor 2A	Cell cycle regulation	56 (30/54)	Chile, USA
UCHL1	Ubiquitin carboxyl terminal esterase L1	Peptidase C12 family	27 (6/22)	Korea
P73	Tumor protein p73	Induction of apoptosis and cell cycle regulation	28 (15/54)	Chile, USA
FHIT	Fragile histidine triad gene	Regulation of DNA replication and apoptosis	30 (6/20) 66 (33/50) 32 (8/25) 57 (13/23)	Chile
DAPK1	Death-associated protein kinase 1	Serine-threonine kinase	22 (2/9) 8 (4/50) 61 (14/23	Japan Chile Chile
RASSF1	RAS association domain family protein 1A	Signal transduction	11 (1/9) 0 (0/50) 8 (4/50) 36 (8/22)	Japan Chile Chile Korea
DLC1	Deleted in liver cancer 1	GTPase-activating protein	39 (9/23)	Chile
p15	Cyclin-dependent kinase inhibitor 2B	Cell cycle regulation	44 (22/50) 22 (5/23)	Chile Chile
SOCS-1	Suppressor of cytokine signaling 1	JAK-STAT pathway	12 (6/50)	Chile
MGMT	O-6-methylguanine-DNA methyltransferase	Methyltransferase	13 (7/54)	Chile, USA

Telomeres

The chromosome ends play an important role in ensuring chromosome stability was first proposed in the 1930s by Barbara McClintock working with maize (McClintock, 1939) and Hermann Muller working with fruit flies (Muller, 1938). Both investigators proposed that chromosome ends have special structures required for chromosome stability. Muller coined the term *telomere*, from the Greek for "end" (*telos*) and "part" (*meros*). McClintock noted that without these special end structures, chromosomes would fuse and often break upon mitosis, and she observed that the resulting chromosome instability was detrimental to cells. These pioneering studies established that functional "telomeres" are required to protect chromosome ends, to provide chromosome stability, and to ensure faithful segregation of genetic material into daughter cells upon cell division.

Telomere structure and functional roles of the telomeric proteins

Telomeres contain a double-stranded region of TTAGGG repeats and a 150–200 nucleotide-long single strand of the G-rich strand. The G-strand overhang (grey strand) invades the doubled stranded DNA region of the telomere to form a protective telomere T-loop, with a displacement D-loop at the invasion site (de Lange, 2005). Mammalian telomeres solve the end protection problem through the agency of six subunit protein complex called shelterin. The shelterin complex binds to the telomere in a T-loop configuration. This complex is composed of telomeric repeat binding factor 1 (TRF1; also known as TERF1), TRF2 (also known as TERF2), repressor-activator protein 1 (RAP1; also known as TERF2IP1), the protection of telomeres protein 1 (POT1), TIN2 (also known as TIFN2) organizing protein (TPP1; also known as ACD), TIN2 and POT1 (de Lange, 2005). TRF1, TRF2 and POT1 bind directly to telomeric DNA repeats, with TRF1 and TRF2 bind to TTAGGG telomeric double-stranded DNA sequence and POT1 to the 3′ singled-stranded G-overhang (Court *et al.,* 2005). TIN2 binds TRF1 and TRF2 through independent domains and recruits the TPP1–POT1 complex, constituting the bridge among the different shelterin components (Chen *et al.,* 2008). Telomerase is a two-partner enzyme, the catalytic subunit (TERT) and the RNA template (*Terc*), which recognizes the hydroxyl group (OH) at the 3′ end of the G-strand overhang and elongates the telomere (Blackburn 2001).

Telomeric chromatin is also enriched in epigenetic marks that are characteristic of constitutive heterochromatin, such as histone tri-methylation and DNA hypermethylation, which act as negative regulators of telomere length and telomere recombination (Blasco, 2007). Telomere shortening below a certain threshold length and/or alterations in the functionality of the telomere-binding proteins can result in loss of telomeric protection, leading to end-to-end chromosome fusions, cell cycle arrest and/or apoptosis. Telomeres also perform other functions, which include the transcriptional silencing of genes located close to the telomeres (this phenomenon is termed subtelomeric silencing), as well as ensuring correct chromosome segregation during mitosis.

Telomeres are thought to be maintained by at least two mechanisms: telomerase activity and recombination. Telomerase synthesizes telomeres at the chromosome ends and thus regulates the length of telomeric repeats (Nakayama *et al.,* 1988; Feng *et al.,* 1995). Telomere length, in some cases, is maintained by an alternative mechanism, such as a telomerase independent telomere length mechanism (Alternative Lengthening of Telomeres or ALT) based on homologous recombination-mediated DNA replication among telomeric sequences (Muntoni *et al.,* 2005).

Telomere Dysfunction as a Driver of Genomic Instability

Telomeric DNA damage originates from various mechanisms. Oncogene-induced replicative stress may cause telomere loss owing to the intrinsic telomere shortening that is associated with replication, as well as an elevated incidence of stalled replications forks at telomeres that resemble fragile site (Martinez *et al.,* 2009; Sfeir *et al.,* 2009; Verdun *et al.,* 2006; McNees *et al.,* 2010). Mutations in telomerase and in shelterin components may result in either telomere loss or severe telomere uncapping (Martinez *and Blasco,* 2010; de lang., 2009). Telomeric DNA is highly susceptible to genotoxic damage (Rochette *et al.,* 2010). Dysfunctional telomeres, either owing to critically short telomeres or to uncapping, elicit a DNA damage response (DDR) by activation of upstream kinases, DNA-dependent protein kinase catalytic subunit (DNA-PKcs), ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) (de lange., 2009). DDR activation may drive cells towards two opposing outcomes depending on the p53 and p21 status. Consequent activation of the tumor suppressor p53 induces cell cycle arrest, apoptosis or senescence, negatively affecting stem cell functionality and causing tissue degeneration and

ultimately organ failure. In *Trp53*-deficient cells, the damage proceeds unchecked and no cell cycle arrest and senescence or apoptotic response will take place. The activation of the ATM–ATR kinase pathways leads to mitotic block. The cells are then able to bypass mitosis and re-enter S phase of the cell cycle, becoming tetraploid (Davoli *et al.,* 2010). Tetraploidization can readily initiate genomic instability owing to the presence of multiple centrosomes that will give rise to the random distribution of chromosome originating aneuploid daughter cells in mitosis. Activation of either the classic or the alternative non-homologous end joining (NHEJ) pathways results in end-to-end fusions that initiate successive cycles of breakage–fusion–bridges. On telomere healing, either by telomerase reactivation or by homologous recombination-based mechanisms, such as the alternative lengthening of the telomere (ALT), stable malignant clones will be generated, giving rise to metastasic tumor

Association of Subtelomeric DNA Methylation with Telomeric Length and Cancer Progression

DNA methylation is one of the epigenetic chromatin modification process, and it plays important roles in gene expression and imprinting, as well as in heterochromatin assembly (Esteller, 2003). DNA methylation is mediated by DNA methyltransferase (DNMTs) known as DNMT1, DNMT3a, and DNMT3b, and three DNMTs have been identified in both humans and mice (Chen *et al.,* 2003).These DNMTs play an important role in maintaining DNA methylation patterns at the pericentric major satellites of heterochromatin, ensuring proper centromeric function (Dodge *et al.,* 2005; Lehnertz *et al.,* 2003).

Subtelomeres are DNA sequences placed between chromosome specific region and chromosomes ends with features that distinguish them from the rest of the genome (Efford and Trask, 2002). Human subtelomeres vary in size from 10 to 500kb in human cells. However, this region is prone to recombination and rearrangement and human disorder. In human populations, the subtelomeric region is highly polymorphic and rate of recombination at chromosome end is higher than in rate of the genome. Such rearrangements participate in the genome variability and the length of variation may be up to hundreds of kilobases among different haplotypes. Various tandemly repeated units, called telomeric associated repeats (TAR-1), short native telomeric array and numerous degenerate telomere like repeats are also located at variable distance from the telomere. Subtelomeres contain members of 25 small families of genes, encoding potentially functional proteins. Telomeres do not contain genes and the CpG sequence that is these are susceptible to methylation by DNMTs, whereas subtelomeres are gene-poor and have a high density of CpG sequences (Blasco, 2007; Steinert *et al.,* 2004).

A role for DNA methylation at the subtelomere has recently been reported in mice; with the discovery of hypermethylated mouse subtelomeric DNA (Benetti *et al.,* 2007; Gonzalo *et al.,* 2006). De-methylation of subtelomeric regions in DNMT-deficient cells results in telomere lengthening caused by increased homologous recombination in telomeric sequences. (Gonzalo *et al.,* 2006).Reintroduction of DNMTs 3a and 3b into DNMT-deficient cells restores methylation at the subtelomere and results in less telomeric homologous recombination. In telomerase knock-out mouse cells, decreased DNA methylation is a consequence of telomere shortening (Benetti *et al.,* 2007). DNA methylation at the subtelomere is therefore implicated as an important regulator of telomeres, raising the possibility that DNA methylation levels might have a close association with telomere length.

Subtelomeric DNA Methylation in Hepatocellular Carcinomas

The subtelomeric DNA methylation status of 7q, 8q, 17q, 18p, 21q and XpYp in Hepatocellular carcinomas (HCCs) and their adjacent non-HCC and it was found that high levels of methylation ratio were found on chromosomes 7q, 18p and XpYp, whereas 8q, 17q and 21q were less methylated. The methylation ratio of 7p, 18p and of 21q was negatively and positively correlated with telomere length of HCCs, respectively. It was analysed that methylation changes proceeded towards hypomethylation as telomere lengthened from non-HCCs to HCCs. Conversely, towards hypermethylation in 21q as telomere lengthened. In summary, subtelomeric methylation at certain regions was related to telomere lengthening or shortening, suggesting an association between subtelomeric chromatin structure and telomere length regulation in human hepatocarcinogenesis (Bong-Kyeong *et al*., 2010).

DNA Methylation of Subtelomeric Tandem Repeats in Bladder Cancer

The level of DNA methylation in subtelomeric tandem repeats of Sat-a and NBL-2 significantly decreased from 71.6% to 58.2% and from 83.2%to 67.3% respectively and that of D4Z4 increased from 51.3% to 58.1% in bladder cancer in comparison to adjacent non tumor tissue (Soussa

et al., 2009). These results were consistent with previous reports that showed D4Z4 elements are hypermethylated in some cancers (Tsumagari *et al*., 2008). This presented the possibility that these elements can be used as an early biomarker to predict cancer.

DNA Methylation Changes in Subtelomeric Tandem Repeats in Leukemia

Both NBL-2 and D4Z4 methylation increased in APL (Acute Promyelocytic Leukemia) and CML (Chronic Myelogenous Leukemia) progression; the increase was slightly greater in APL. Thus, the methylation of NBL-2 decreases in bladder tumor progression but increases during leukemogenesis (Choi *et al*., 2009). These findings were consistent with the previous reported, showing that NBL-2 repeats can be either hypomethylated in neuroblastoma and hepatocellular carcinoma or hypermethylated in ovarian epithelial carcinomas (Nishiyama *et al*., 2005). Nishiyma *et al* (2005) also reported that hyper- and hypomethylation at different individual CpG sites within same NBL-2 repeat in ovarian cancer. This indicates that the behavior of DNA methylation of subtelomeric tandem repeats may vary from cancer to cancer and deserves further investigation.

Methylation of D4Z4 Repeat in Ovarian Epithelial Carcinomas and Wilms Tumor

The ovarian epithelial carcinomas and Wilms tumors displayed large differences in D4Z4 methylation; most specimens from both of these diverse types of cancers were either significantly hypo or hypermethylated at D4Z4 EagI and SmaI sites relative to somatic control tissues respectively. Several of the cancers had extremely high levels of methylation in consecutive 3.3-kb repeat units of D4Z4 array For example, the amount of D4Z4 double digested with the CpG methylation insensitive KpnI was 52–88% for Wilms tumor (WT) as compared with 7–14% for control brain DNA and In single digests of WT DNA with BstUI or HpyCH4IV, most of the D4Z4 signal was in fragments of more than 10 kb suggesting that methylation spreads along the arrays during normal development and during tumorgenesis with long regions of complete methylation and interspersed regions of considerably lower methylation (Tsumagari *et al*., 2008).

Subtelomeric Methylation in ALT Cells and Telomerase-Positive Cells

The subtelomeric methylation at 2p, 4p and 18p loci in human tumor derived cell lines that use either ALT or telomerase as the telomere maintenance mechanism. The ALT tumor cell lines displayed highly heterogeneous patterns of subtelomeric methylation. In contrast to ALT cells, telomerase-positive tumor cell lines invariably showed dense methylation (97%) at all subtelomeric loci examined and had significantly more subtelomeric methylation than ALT cells and normal PBMC.

The telomeric repeat-containing transcript TERRA (Telomeric Repeat Containing-RNA) is transcribed from the C-rich strand using promoters situated in the subtelomeric region. In telomerase-positive cells, low TERRA transcript levels and high levels of subtelomeric methylation reflects selection for TERRA silencing in order to facilitate telomerase activity at the telomere. These data suggests that the epigenetic differences between telomerase positive and ALT cells may underlie the mechanism of telomere maintenance in human tumorgenesis (Laura *et al*., 2009).

Subtelomeric DNA Methylation in Human Cancer Cell Lines

The subtelomeric DNA methylation was determined by Lee *et al*., 2010 for seven regions on five chromosomes (2, 9, 10, 17 and 21) in 20 human cancer cell lines. Full-, partial- and un-methylation patterns were found at frequencies of 39% (55/140), 36% (50/140), and 25% (35/140), respectively, suggesting that various methylation patterns were present at the subtelomeres. Methylation was dominant at 9q (84 kb) and 17q (13 kb) and unmethylation was dominant at 9p (18 kb), suggesting that subtelomeric DNA methylation patterns might differ according to the location of CpG sites and certain subtelomeric regions might maintain a fixed (invariable) chromatin structure.

The frequency of full-, partial-, and un-methylation was 31% (22/70), 41% (29/70), and 27% (19/70) in the short telomere group and 47% (33/70), 30% (21/70), and 23% (16/70) in the long telomere cell group respectively and the full-methylation tended to be more prevalent in the long telomere group compared to the short telomere group (Lee *et al*., 2010).

REFERENCES

Albores-Saavedra J, Wadaji M, Henson DE, Ziegels-Weissman J and Mones JM. (1986). Intestinal metaplasia of the gallbladder: A morphological and immunocytochemical study. *Hum Pathol* **17:** 614-620.

Albores-Saavedra J and Henson DE. Tumors of the gallbladder and extrahepatic bile ducts: Atlas of tumor pathology. Fas, 22, ser. 2, Washington, D.C: Armed forces institute of pathology.

Aldridge MC and Bismuth H. (1990). Gallbladder cancer: The polyp-cancer sequence. *Br J Surg* **77:** 363364.

Allis CD, Jenuwein T and Reinberg D. (2007). Epigenetics edn. *Genetics Research* **89**(2): 124-125

Artico M, Bronzetti E, Alicino V, Ionta B, Bosco S, *et al*. (2010). Human gallbladder carcinoma: Role of neurotrophins, MIB-1, CD34 and CA15-3. *European Journal of Histochemistry* **54:** 10.

Arundhati R, Mohapatra SC and Shukla HS. (2004). A review of association of dietary in gall bladder. *Ind Journal of Cancer* **16:** 41-52.

Bartlett DL. (2000). Gall bladder cancer scenario. *Surg Oncol* **19:** 145-55.

Baylin S. (2001). DNA methylation and epigenetic mechanisms of carcinogenesis. *Dev Biol* **106:** 85-87.

Benetti R, Garcia-Cao M and Blasco MA. (2007). Telomere length regulates the epigenetic status of mammalian telomeres and subtelomeres. *Nat Genet* **39:** 243-250.

Bird AP. (1986). CpG-rich islands and the function of DNA methylation. *Nature* **321:** 209-13.

Blasco MA. (2007). The epigenetic regulation of mammalian telomeres. *Nat Rev Genet* **8:** 299-309.

Bong-Kyeong OH, Tae-Hee UM, Choi GH and Park YN. (2010). Frequent changes in subtelomeric DNA methylation patterns and its relevance to telomere regulation during human Hepatocarcinogenesis. *Int J Cancer* **128:** 857-868.

Boss JL. (1988). The ras gene family and human carcinogenesis. *Mutant Res* **195:** 255-271.

Calle EE, Rodriguez C, Walker-Thurmond K and Thun MJ. (2003). Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* **348:**/1625-38.

Chao TC, Wang CS, Jan YY, Chen HM and Chen MF. (1999). Carcinogenesis in the biliary system associated with APDJ. *J Hepatobiliary Pancreat Surg* **6:** 218-222.

Chen T, Ueda Y, Dodge JE, Wang Z and Li E. (2003). Establishment and maintenance of genomic methylation patterns in mouse embryonic stem cells by DNMT 3a and DNMT 3b. *Mol Cell Biol* **23:** 5594-5605.

Chen Y. *et al.* (2008). A shared docking motif in TRF1 andTRF2 used for differential recruitment of telomeric proteins. *Science* **319:** 1092-1096.

Choi SH, Worswick S, Byun HM, Shear T, Soussa JC, *et al*. (2009). Changes in DNA methylation of tandem DNA repeats are different from interspersed repeats in cancer. *Int J Cancer* **125:** 723-729.

Chow WH, Johansen C, Gridley G, Mellemkjaer L, Olsen JH and Fraumeni JF Jr. (1999). Gallstones, cholecystectomy and risk of cancers of the liver, biliary tract and pancreas*. Br J Cancer* **79:** 640-644.

Chuang JC and Jones PA. (2007). Epigenetics and microRNAs. *Pediatr Res* **61:** 24-24.

Curado MP, Edwards B and Shin HR. (2007). Cancer Incidence in Five Continents, International Agency for Research Cancer, Lyon, France, *Scientific Publications* **9:** 160.

Davoli T, Denchi EL and de Lange T. (2010). Persistent telomere damage induces bypass of mitosis and tetraploidy. *Cell* **141:** 81-93.

de Lange T. (2005). Shelterin: The protein complex that shapes and safeguards human telomeres. *Genes Dev* **19:** 2100-2110.

de Lange T. (2009). How telomeres solve the end-protection problem. *Science* **326:** 948-952.

Deng Y and Chan SS. (2008). Telomere dysfunction and tumor suppression: The senescence connection. *Nat Rev Cancer* **8(6):** 450-8.

Diehl AK. (1983). Gallstone size and the risk of gallbladder cancer. *JAMA* **250:** 2323-2326.

Dodge JE, Okano M, Dick F, Tsujimoto N, Chen T, *et al.* (2005). Inactivation of DNMT 3b in mouse embryonic fibroblasts results in DNA hypomethylation, chromosomal instability, and spontaneous immortalization, *J Biol Chem* **280:** 17986-17991.

Esteller M and Herman JG. (2002). Cancer as an epigenetic disease: DNA methylation and chromatin alterations in human tumors. *J Pathol* **196:** 1-7.

Esteller M. (2003). Relevance of DNA methylation in the management of cancer, *Lancet Oncol* **4:** 351-358.

Feng J, Funk WD, Wang SS, Weinrich SL, Avilion AA, *et al*. (1995). The RNA component of human telomerase. *Science* **269:** 1236-1241.

Frierson H. (1997). Gallbladder and extrahepatic biliary system. In Stenberg S (ed.) Histopathology for pathologist ed.2 Philidelphia, Lippinett- Ranen publishers, 593-612.

Garcia P, Manterola C, Araya JC, Villaseca M, Guzman P, *et al*. (2009). Promoter methylation profile in preneoplastic and neoplastic gallbladder lesions. *Mol Carcinog* **48:** 79-89.

Garcia-Aranda C, de Jaun C, Diaz- Lopez A, Sanchez-Pernaute A, Torres AJ, *et al*. (2006). Correlation of telomere length, telomerase activity, and telomeric repeat binding factor 1 expression in colorectal carcinoma. *Cancer* **106:** 541-551.

Germain M, Martin E and Grenillet C. (1979). Procelain gallbladder and cancer. *Author Transl Hop* **55:** 1629-1632.

Gonzalo S, Jaco I, Fraga MF, Chen T, Li E, Esteller M and Blasco MA. (2006). DNA methyltransferase control telomere length and telomere recombination in mammalian cells. *Nat Cell Biol* **8:** 416-424

Gourgiotis S, Kocher HM, Solaini L, Yarollahi A, Tsiambas E and Salemis NS. (2008). Gallbladder cancer. *Am. J. Surg.,* **196:** 252-264.

Green Blatt MS, Bennett WP, Hollatein M and Harris CC. (1994). Mutation in p53 tumor suppressor gene; clue to cancer etiology and molecular pathogenesis. *Cancer Res.,* **54:** 4855-4878.

Grewal SI and Moazed D. (2003). Heterochromatin and epigenetic control of gene expression. *Science* **301:** 798-802.

Hai AA, Sinha DN and Shah HC. (1994). Possible etiology Of gallbladder carcinoma, XVI International Cancer Congress ed., Monduzii. 2069-72

Hanada K, Tsuchida A, Iwao T, Sasaki T *et al.* (1999). Gene mutation of K-ras in gallbladder moucosa and gallbladder carcinoma with APJBD. *Am J Gastroenternol* **94:** 1638-1642.

House MG, Wistuba II, Argani P, Guo M, Schulick RD, *et al*. (2003). Progression of gene hypermethylation in gallstone disease leading to gallbladder cancer. *Ann Surg Oncol* **10:** 882-889.

Hsing AW, Bai Y, Andreotti G *et al.* (2007). Family history of gallstones and the risk of biliary tract cancer and gallstones: A population based study in Shanghai, China. *Int J Cancer* **121:** 832-838.

Ishibashi Y, Takada K, Joh K, Ohkawa K, Aoki T and Matsuda M. (1991). Ubiquitin immunoreactivity in human malignant tumors. *Br J Cancer* **63:** 320-322.

Jackson RJ and Standart N. (2007). How do microRNAs regulate gene expression? *Sci STKE* **250:** 151-342.

Kang CM, Kim KE, Choi JS and Lee WJ. (2007). Gallbladder carcinoma associated with APJBD. *J Gastroenteric* **21:** 383-387.

Kapoor VK and McMichael AJ. (2003). Gallbladder cancer: An 'Indian' disease. *Natl Med J India* **16:** 209-13.

Kee SK, Lee JY, Kim MJ, Lee SM, Jung YW, *et al*. (2007). Hypermethylation of the Ras Association Domain Family 1A (RASSF1A) gene in gallbladder cancer. *Mol Cell* **24:** 364-371.

Kim YW, Huh SH, Park YK, Yoon TY, Lee SM and Hong SH. (2001). Expression of the c-erb-B2 and p53 protein in gallbladder carcinomas. *Oncol Rep* **8:** 1127-1132.

Kuroki T, Tajima Y, Matsuo K and Kanematsu T. (2005). Genetic alterations in gallbladder carcinoma. *Surg Today* **35:** 101-105.

Laitio M and Nevalainen T. (1975). Gland ultrastructure in human gallbladder. *J Anat* **120:** 105-112.

Laitio M. (1980). Morphology and histochemistry of non tumorous gallbladder epithelium: A series of 1033 cases. *Pathol Res Pract* **167:** 335-345.

Larsson SC and Wolk A. (2007). Obesity and the risk of gallbladder cancer: A meta-analysis. *Br J Cancer* **96:** 1457-1461.

Laurah M, Karanjia NP and Dickson GH. (2001). Anatomical variations of the extrahepatic billary tree: Review of world literature. *Clin Anat* **14:** 167-172.

Lazcano-Ponce EC, Miquel JF, Munoz *et al.* (1985). Epidemiology and mol. pathology of gall bladder. *J Natl Cancer Inst* **75:** 77-80.

Lazcano-Ponce EC, Miquel JF, Munoz N, Herrero R, Ferrecio C, *et al.* (2001). Epidemiology and molecular pathology of gallbladder cancer. *CA Cancer. J Clin* **51:** 349-364.

Lee ME, Rha SY, Jeung HC, Kim TS, Chung HC and Oh BK. (2006). Variation of the 30 telomeric overhang lengths in human cells. *Cancer Lett* **264:** 107-118.

Lehnertz B, Ueda Y, Derijck AA, Braunschweig U, Perez-Burgos L, *et al*. (2003). SUV-39-mediated histone H3 lysine methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. *Curr Biol* **13:** 1192-1200.

Letelier P, Tapia PB and Roa JC. (2012). DNA promoter methylation as a diagnostic and therapeutic biomarker in gallbladder cancer. *Clinical Epigenetics* **4:** 11.

Levin AJ. (1997). The cellular gene keeper for growth and division. *Cell* **88:** 323-331.

Levy AD, Murakata LA and Rohrmann CA, Jr. (2001). Gall bladder carcinoma: Radiological and pathologic correlation. *Radiographics* **21:** 295-314.

Liggett WH Jr and Sidransky D. (1998). Role of the p16 tumor suppressor gene in cancer. *J Clin Oncol* **16:** 1197–1206.

Liu Y, Fallon L, Lashuel HA, Liu Z and Lansbury PT, Jr. (2002). The UCH-L1 gene encodes two opposing enzymatic activities that affect alpha-synuclein degradation and Parkinson's disease susceptibility. *Cell* **111:** 209-218.

Lowenfels AB, Lindstrm CG, Conway MJ and Hastings PR. (1985). Gallstones and risk of gallbladder cancer. *J Natl Cancer Inst* **75:** 77-80.

Macarthur M, Hold GL and El-Omar EM. (2004). Inflammation and Cancer II. Role of chronic inflammation and cytokine gene polymorphisms in the pathogenesis of gastrointestinal malignancy. *Am J Physiol Gastrointest* **286:** 515-520.

Martinez P and Blasco MA. (2010). Role of shelterin in cancer and aging. *Aging Cell* **9:** 653-666.

Martinez P. *et al.* (2009). Increased telomere fragility and fusions resulting from TRF1 deficiency lead to degenerative pathologies and increased cancer in mice. *Genes Dev* **23:** 2060–2075.

McClintock B. (1939). The behavior in successive nuclear divisions of a chromosome broken at meiosis. *Proc Natl Acad Sci USA* **25:** 405-416.

McNees CJ. *et al.* (2010). ATR suppresses telomere fragility and recombination but is dispensable for elongation of short telomeres by telomerase. *J Cell Biol* **188:** 639-652.

Michael G, House MD, Ignacio I, Wistuba MD, Pedram Argani MD, *et al*. (2003). Progression of Gene Hypermethylation in Gallstone Disease Leading to Gallbladder Cancer. *Annals of Surgical Oncology* **10(8):** 882-889.

Millar D, Holliday R and Grigg G. (2003). Five not four: History and significance of the fifth base.The Epigenome, Molecular Hide and Seek. Wiley-VCH Verlag GmbH & Co. KGaA, 3-20.

Misra S, Chaturvedi A, Misra NC and Sharma ID. (2003). Carcinoma of the gallbladder. *Lancet Oncol* **4:** 167-176.

Moerman CJ, Bueno de Mesquita HB, Smeets FW and Runia S. (1995). Consumption of foods and micronutrients and the risk of cancer of the biliary tract. *Prev Med* **24:** 591-602.

Muller HJ. (1938). The re-making of chromosomes. *Collecting Net, Woods Hole* **13:** 181-198.

Muntoni A and Reddel RR. (2005). The first molecular details of ALT in human tumor cells. *Hum Mol Genet* **14:** 191-196.

Nakayama J, Tahara H, Tahara E, Saito M, Ito K, *et al*. (2008). Telomerase activation by hTERT in human normal fibroblasts and hepatocellular carcinomas. *Nat Genet* **18:** 65-68.

Nath G, Sngh YK, Maurya P and Gulati AK. (2010). Does *Salmonella typhii* primarily resides in liver chronic typhoid carriers. *J Infect Dio Ctries* **4:** 259-62.

National Cancer Registry Programme. (2001). Consolidated report of the population based cancer registries 1990-1996. *Indian Council of Medical Research* New Delhi, India. 52-3.

Nishiyama R, Qi L, Tsumagari K, Weissbecker K, Dubeau L, Champagne M, Sikka S, Nagai H and Ehrlich

M. (2005). A DNA repeat, NBL2, is hypermethylated in some cancers but hypomethylated in others. *Cancer Biol Ther* **4:** 440–8.

Nuzzo G, Clemente G, Cadeddu F, Ardito F, Ricci R and Vecchio FM. (2005). Papillary carcinoma of the gallbladder and anomalous pancreatico-biliary junction. Report of three cases and review of the literature. *Hepatogastroenterology* **52:** 1034-1038.

Orphanides G and Reinberg D. (2002). A unified theory of gene expression. *Cell* **108:** 439-451.

Pandey M and Shukla VK. (2003). Lifestyle, parity, menstrual and reproductive factors and risk of gallbladder cancer. *Eur J Cancer Prev* **12:**/269-72.

Pandey M, Shukla M and Shuka V. (2008). Diet and gallbladder cancer. *Ind J of Med and Pediatric Oncology* **29(I):** 6-7.

Pandey M. (2006). Environmental pollutants in gallbladder carcinogenesis. *J Surg Oncol* **93:** 640–643.

Piehler JM and Crichlow RW. (1978). Primary carcinoma of the gallbladder. A review of 328 cases. *Surg Gynecol Obstet* **147:** 929.

Pillai RS, Bhattacharyya SN and Filipowicz W. (2007). Repression of protein synthesis by miRNAs: How many mechanisms? *Trends Cell Biol* **17:** 118–126.

Randi G, Franceschi S and La Vecchia C. (2006). Gallbladder cancer worldwide: Geographical distribution and risk factors. *Int J Cancer* **118:** 1591–1602.

Reik W, Dean W and Walter J. (2001). Epigenetic reprogramming in mammalian development. *Science* **293:** 1089-1093.

Riquelme E, Tang M, Baez S, Diaz A, Pruyas M, Wistuba II and Corvalan A. (2007). Frequent epigenetic inactivation of chromosome 3p candidate tumor suppressor genes in gallbladder carcinoma. *Cancer Lett* **250:** 100–106.

Roa I, Araya JC, Wistuba I, Villaseca M, de Aretxabala X and Burgos L. (1994). Gallbladder cancer in the IX Region of Chile. Impact of the anatomopathological study of 474 cases. *Rev Med Chile* **122:** 1248–1256.

Rochette PJ and Brash DE. (2010). Human telomeres are hypersensitive to UV-induced DNA Damage and refractory to repair. *PLoS Genet* **6:** 1223-1256.

Sasatomi E, Tokunga D and Miyazaki K. (2000). Pre cancerous condition of gall bladder carcinoma: overview of histopathogic characteristics and molecular genetics findings. *J Hepatobiliary Pancreat Surg* **7:** 556-567.

Sfeir A. *et al.* (2009). Mammalian telomeres resemble fragile sites and require TRF1 for efficient replication. *Cell* **138:** 90-103.

Steinert S, Shay JW and Wright WE. (2004). Modification of subtelomeric DNA. *Mol Cell Biol* **24:** 4571-4580.

Sumiyoshi K, Nagai E, Chijiiwa K and Nakayama F. (1991). Pathology of carcinoma of the gallbladder. *World J Surg* **15:** 315-21.

Tadokoro H, Shigihara T, Ikeda T, Takase M and Suyama M. (2007). Two distinct pathways of p16 gene inactivation in gallbladder cancer. *World J Gastroenterol* **13:** 6396-6403.

Towfigh S, McFadden DW, Cortina GR *et al.* (2001). Porcelain gallbladder is not associated with gallbladder carcinoma. *Am Surg* **67:** 7-10.

Tozawa T, Tamura G, Honda T, Nawata S, Kimura W *et al.* (2004). Promoter hypermethylation of DAP-kinase is associated with poor survival in primary biliary tract carcinoma patients. *Cancer Sci* **95:** 736-740.

Tsumagari K, Qi L, Jackson K, Shao C, Lacey M, Sowden J *et al.* (2008). Epigenetics of a tandem DNA repeat: Chromatin DNaseI sensitivity and opposite methylation changes in cancers. *Nucleic Acids Res* **36:** 2196-2207.

Verdun RE and Karlseder J. (2006). The DNA damage machinery and homologous recombination pathway act consecutively to protect human telomeres. *Cell* **127:** 709-720.

Vitetta L, Sali A, Little P and Mrazek L. (2000). Gallstones and gall bladder carcinoma. *Aust NZ J Surg* **70:** 667-673.

Wistuba II and Gazdar AF. (2004). Gallbladder Cancer: Lessons from a rare tumour. *Nature Review* **4:** 697.

Zatonski WA, Lowenfels AB, Boyle P *et al.* (1997). Epidemiologic aspects of gallbladder cancer: A case control study of the search Program of the International Agency for Research on Cancer. *J Nat Cancer Inst* **89:** 1132-1138.

Zielenski MD, Atwell TD and Davis PW. (2009). Comparison of surgically resected polypoid lesions of the gall bladder to their pre-operative ultrasound characteristics. *J Gastrointest Surg* **13:** 19-25.