

Review Article

Molecular genetics changes in gallbladder carcinoma

Khushi Verma¹, Ruhi Dixit¹, Juhi Singh², Satyendra K. Tiwary¹, Ajay K. Khanna¹, Gopeshwar Narayan³, Puneet Kumar¹

Departments of ¹General Surgery, ²Gastroenterology and ³Molecular and Human Genetics, Institute of Medical Sciences, Banaras Hindu University, Varanasi, Uttar Pradesh, India.



***Corresponding author:**

Dr. Puneet Kumar,
Department of General Surgery,
Institute of Medical Sciences,
Banaras Hindu University,
Varanasi, Uttar Pradesh -
221005, India.

puneetimsbhu@gmail.com

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ABSTRACT

Gallbladder carcinoma (GBC) is the fifth most common cancer of gastrointestinal tract with higher preponderance in women. This tumor is a highly aggressive disease with an overall 5-year survival of <5%. The disease presents in advanced stages where the treatment is ineffective. Different causative factors are documented to be linked with GBC pathogenesis such as environmental, microbial, metabolic, and molecular. One of the most important causing factors is molecular genetic alterations which are associated with GBC. Genetic mutations can be germline or somatic that involving proto-oncogenes, tumor suppressor genes, cell cycle, and growth factors. Several researches have been done to identify altered genes responsible for gallbladder carcinogenesis but the exact mechanism behind molecular pathogenesis still needs to be spread out. In this review, we summarized some of the important genetic changes reported in GBC to date that can be explored to achieve better understanding in gallbladder carcinogenesis.

Keywords: Gallbladder carcinoma, Oncogenes, Tumor suppressor genes, Cell cycle regulators, Adhesion molecules

INTRODUCTION

The first description of the gallbladder carcinoma (GBC) was given by Maxmillan de Stol in 1777. GBC is a very lethal and aggressive of the biliary cancers with a poor prognosis and the shortest median survival duration.^[1-5] The symptoms and signs of GBC are vague and the clinical presentation is nonspecific may include abdominal pain, weight loss, fever, and jaundice, and any of these can be found in cholecystitis.^[6-8] Most cancers originate in the fundus (fundus [60%], body [30%], or neck [10%]).^[9] GBC has been reported to be 3–5 times more common in females than males.

The frequency of GBC progressively increases with age, even though it varies significantly in different parts of the world and also incidence varies within a country.^[3,10-13] In India, the frequency rate of GBC is greatly higher in the North (e.g., incidence in Delhi is 4.5/100,000 for men and 10.1/100,000 for women) as compared with the South (e.g., in Chennai, Mumbai, Trivandrum, and Bengaluru incidence is 1.2/100,000 for men and 0.9/100,000 for women).^[3,13] Histologically, the predominance of GBC is adenocarcinomas (80–95%),^[14,15] and the leftover less common types include undifferentiated or anaplastic carcinoma (2–7%), squamous cell carcinoma (1–6%), and adenosquamous carcinoma (1–4%).^[3,4,6,16-18]

The etiology of the disease is influenced by several risk factors which include exposure to certain chemicals and heavy metals, age, female gender, family history, obesity, cholelithiasis, chronic cholecystitis, porcelain gallbladder, polyps, and chronic infection by salmonella species along with

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various genetic factors.^[3,14,15,19,20] Molecular genetic factors play an important role in gallbladder carcinogenesis.

GENETIC AND MOLECULAR CHANGE

GBC is a multi-factorial disease involving numerous genetic changes. In view of the present knowledge, genetic and molecular changes in GBC are still needs to be explored.^[21-24] Some common aberrations include the activation of oncogenes, inactivation of tumor suppressor genes (TSGs), presence of microsatellite instability, and epigenetic changes mainly caused by atypical promoter methylation of gene areas are the various common factors reported till now. The serious of genetic changes leading to gallbladder cancer development is still not well known.^[21,25] The present article will describe some of the important genetic changes reported in GBC [Table 1].

Oncogenes

Oncogenes cause alteration in normal cells to transform it into cancerous cells (with abnormal cell growth) is considered as oncogenes. Oncogenes are usually mutated forms of normal cellular genes (proto-oncogenes) that get altered in the gallbladder cancer signaling cascade. When a proto-oncogene altered, it becomes a “gain of function mutant gene” that can become permanently activated and influence cancer formation.^[26]

K-*ras*

The *ras* gene family is a group of three closely associated cellular proto-oncogenes which play an significant role in the etiology of human cancers. There are three members of the *ras* family, namely N-*ras*, H-*ras*, and K-*ras* genes.^[27] Many pathogenic mutations have been described in K-*ras* oncogene in GBC tissue.^[21,28-30] K-*ras* encodes a 21-kD protein that is situated on the inner side of the plasma membranes that are a small GTP-binding cytoplasmic protein that has intrinsic GTPase activity and hydrolyzes guanosine triphosphate to guanosine diphosphate. K-*ras* protein that is bound to guanosine triphosphate is active, involved in signal transduction, and molecular switches in the cell cycle.^[24,31,32] K-*ras* gene mutations identified in GBC generally affects codons 12, 13, and 61^[21,33] results in inability to hydrolyze bound guanosine triphosphate and incapability to switch back to the inactive configuration, resultant inappropriate growth signal.^[24] Most of the mutations were found in codon 12 of K-*ras* gene, and mutations of codons 13 and 61 were less common.^[24,34-36] In North India, K-*ras* codon 13 mutation is more frequent (about one-third) than codon 12 and 61.^[21,33] The identified rates of K-*ras* mutations were 5–57% for gallbladder cancers.^[24,37-39]

GBC connected with an Anomalous Pancreaticobiliary Duct Junction have a high K-*ras* gene mutation rate in 50–83% of cases and this indicate that the detection of K-*ras* mutation is a valuable molecular diagnostic marker for GBC in this

category of patients.^[33,40,41] Although mutation of K-*ras* gene has never been identified in GBC having adenoma carcinoma sequence of development.^[21,42]

Epidermal growth factor receptor (EGFR)

The EGF RTK family consists of four members: EGFR (ErbB1, and HER1), ErbB2 (HER2, *neu* in rodents), ErbB3 (HER3), and ErbB4 (HER4).^[43-46] EGFR is a transmembrane receptor belonging to the ErbB family of receptor tyrosine kinases consists of an extracellular ligand-binding domain, a transmembrane domain, a short juxtamembrane section, a tyrosine kinase domain, and a tyrosine-containing C-terminal tail have a molecular weight of 170 kd.^[47-49] The mature EGFR is made up of a single polypeptide chain of 1186 amino acid residues and a substantial amount of N-linked oligosaccharide.^[50] There are six mammalian ligands that bind to EGFR, including itself EGF, transforming growth factor- α , amphiregulin, heparin-binding EGF-like growth factor, betacellulin, and epiregulin.^[51]

Ligand binding to the EGFR receptor induces a conformational change of the receptor ectodomain that allows for receptor dimerization and autophosphorylation through tyrosine kinase activity of several tyrosine residues within the COOH-terminal tail of the receptors.^[11,44,52-54] Autophosphorylation causes a chain of intracellular pathways that possibly result in cancer-cell proliferation, blocking apoptosis, activating invasion and metastasis, and stimulating tumor-induced neovascularization.^[55]

EGFR is normally present within the plasma membrane of many types of normal cells, it is commonly over-expressed or over-activated in malignant cells. When active, it stimulates intracellular pathways that promote cell proliferation and angiogenesis, in addition to evasion of apoptosis.^[47] Since the 1980s, a number of reports defined the overexpression of EGFR in a variety of epithelial tumors, supporting the hypothesis that dysregulation of EGFR gene expression and signaling could play a pivotal biological role in cancer.^[56]

In 1995, Lee and Pirdas were the first to report a correlation between the membrane receptor EGFR and cases of GBC and other bile duct tumors, and its association with gallbladder dysplasia and chronic calculous cholecystitis.^[48,57] EGFR overexpressed in gallbladder, common bile duct, and ampullary carcinomas but not in non-malignant situations of the gallbladder and biliary tract^[58] such as in GBCs (63.4% and 70.7%) and dysplasia (71.4% and 85.7%) than in simple hyperplasia (15.4% and 27%) and normal gallbladder (0%).^[40,59] The EGFR mutations, including activating and resistant mutations, frequently occur in exons 18–21 of the EGFR gene encoding the adenosine triphosphate-binding pocket of the intracellular TK domain.^[56] Somatic mutations of the EGFR tyrosine kinase domain are reported in nearly

15% of the GBC.^[58] Therapeutic targeting of EGFR/HER2 pathways increases the anti-proliferative effect of gemcitabine in GBC as shown by an earlier study, this can become a marker for poor prognosis.^[21,60]

HER2/neu gene (ErbB2)

The HER2/neu gene is a proto-oncogene located on the 17q12-q21 chromosomal region and acts as an oncogene in several human cancers.^[61,62] The role of HER family of tyrosine kinases playing an important role in the pathogenesis of cancer is well established. Among the four HER family proteins, HER2 has the strongest catalytic kinase activity and is the favored partner for dimerization with other HER family proteins.^[63] The HER2/neu receptors undergo dimerization and transphosphorylation of their intracellular domains, which trans-activate numerous intracellular signaling molecules and adaptor proteins (Shc), which in turn lead to the activation of downstream pathways, such as *ras*-RAF-MEK ERK1/2 and PI3k-AKT-mTOR resulting in cancer cell proliferation and cell survival.^[63,64] HER-2/neu (ERBB-2) overexpression reported to occur in 15.7%–63.6% cases of GBC. Overexpression of HER-2/neu is important for carcinogenesis of gallbladder cancers and helpful for detection of early diagnosis.^[40]

TSGS

TSGs are cellular genes that regulate cell division, DNA repair, and apoptosis (tell cells when to die/programmed cell death). When TSGs became non-functional, cells could get uncontrolled growth, leads to cancer. Allelic loss of TSGs, as well loss of heterozygosity (LOH) at polymorphic loci becomes the hallmarks of different cancers.^[64]

TP53

TP53 is well-known TSG, situated on the short arm of chromosome 17p13,^[31] encodes a 53-kD nuclear phosphoprotein which acts as a “Guardian of the genome” transcription factor, mainly in response to DNA damage by ultraviolet irradiation, γ -irradiation, and carcinogens^[25,27,65,66] and has numerous mechanisms of anticancer function and plays an important role in maintenance of genome integrity, apoptosis, genomic stability, and inhibition of angiogenesis etc.^[21,67]

TP53 gene is involved in cellular processes, such as gene expression, DNA repair, and apoptosis. Dysregulation in *TP53* gene leads to cancer cell proliferation and immortality. Until the present, targeting *TP53* gene mutations have evidenced to be a challenge. A number of approaches have been proposed: (1) Wee-1 kinase inhibitors together with DNA harmful agents, (2) inhibitors of the *p53*-MDM2 contact, which result in *p53* stabilization, and (3) vascular endothelial growth factor (VEGF) receptor/VEGF-targeting agents in patients with *TP53* mutations.^[68]

Mutations in *TP53* gene are reported in a majority of human malignancies^[69,70] and point mutations of the *TP53* gene are among the greatest common genetic alterations in human cancers and are supposed to perform a critical role in the pathogenesis of many malignancies including GBC.^[27,29,36-38,65,70-72] However, a missense mutation or a deletion of one *p53* allele often consequences in the lack of the wild-type *p53* protein, result in the loss of tumor suppressor function and permits the promotion of carcinogenesis.^[70] Studies of exons 5–8 and exons 9 of the *TP53* gene have been shown point mutations in 27%–70% of GBCs.^[21,27,38,40,73,74] Mutated *p53* proteins have a longer half-life as compared to their wild-type *p53* product.^[25,63,67,75] *TP53* abnormalities have been shown in patients of two regions, i.e., Japan and Chile with a high occurrence of GBCs.^[70,73]

Fragile histidine triad (FHIT)

FHIT is a TSG that lost during gallbladder carcinogenesis, which reported on chromosome 3p. There are four distinct 3p regions (3p22-24, 3p21.3, 3p14.2, and 3p12) found in GBC with frequent LOH and have been reported by refined allele-type analysis.^[31] A high incidence of LOH in the FHIT region was identified in both carcinoma and dysplastic epithelium of the gallbladder.^[31,76] The loss of FHIT prompts global genome instability, mostly through nucleotide imbalance, spontaneous replication stress, and DNA breaks whereas methylation of the promoter, frameshift mutation, and elevated expression are common in GBC. In the gallbladder, FHIT allelic loss was detected in 13% of samples with chronic cholecystitis, 40% of dysplasia, and 76% of invasive carcinomas.^[40,76-78]

Wistuba *et al.* reported the high correlation between immunostaining in the specimen and allelic loss.^[74,76] and also noted that of a reduction in expression of FHIT in GBC.^[74,78] Recent studies have reported that FHIT did not show any important tendency with respect to gallbladder cancer patient's survival.^[21]

Retinoblastoma (Rb) gene

Rb gene is tumor-suppressor gene encodes a protein (pRb) that acts by altering the activity of transcription factors in human Rb. Many types of cancer are mutated by Rb gene. Rb is a gene that has been found to be present in 100% of the patients with cholecystitis and adenoma, while it is deleted in carcinoma of the gallbladder in 18%–67% of the patients. It has been reported the expression of the pRb correlates with stage, grade, and overall survival.^[79] In other study, Rb protein was found to be expressed in 24% of carcinoma, 32% of gallstones, and 50% of normal gallbladders.^[80]

ADHESION MOLECULES

Cell adhesion molecules (CAMs) are proteins by nature, positioned on the cell surface involved in the association

with other cells or with the extracellular matrix (ECM). Diverse CAMs (immunoglobulin such as protein CAM, cadherins, selectins, and integrins) take part in intercellular and cell-ECM interactions in case of cancer. Some adhesion molecules play a crucial role in the development of repeated, invasive, and distant metastatic cancer conditions.^[81]

MUC1

Mucins are glycoproteins with high molecular weight. It provides a protective layer on epithelial surfaces, lubricating roles in the normal epithelium of gastrointestinal organs and significantly altered during neoplastic, paraneoplastic condition and are involved in cell-cell interactions, signaling, and metastasis.^[40,82,83] Normal mucosal cells secrete a variety of different mucins such as MUC1, MUC2, MUC3, MUC4, MUC5A, and MUC6.^[82,83] The MUC1 is located on chromosome 1q21 and MUC2, MUC5AC, and MUC6 on 11p15.5.^[74,84]

MUC1 is a transmembrane associated mucin large cell surface mucin glycoprotein expressed in a variety of cells in most glandular and ductal epithelial cells.^[82] It has been reported that MUC staining on immunohistochemistry mainly in the cytoplasm and/or the cell membrane rather than the nucleus.^[74,85,86] Chang *et al.* characterized the expression profile of MUC1, significantly higher than MUC5AC^[40,87] and MUC6 in GBC. The expression of MUC1 is absent in normal gallbladder mucosa, whereas MUC5AC and MUC6 are much diffused.^[40,88] MUC1 levels are significantly increased in gallbladder cancer, MUC2 and MUC5AC expression levels are reduced in gallbladder cancer when compared with dysplastic tissue as well carcinoma *in situ*, while the findings in the case of the two studies reporting expression levels of MUC6 are contradictory.^[74,82,85-88] Finzi *et al.* have proposed that the MUC5AC is overproduced in gallstone disease by an inflammation-dependent EGFR cascade.^[74,89] Previous studies have revealed that altered or an inappropriate mucin gene expression is related to tumor development, invasiveness, and prognosis.^[87]

Erythrocyte complement receptor 1 (CR1)

Erythrocyte CR1 present on erythrocytes is a membrane protein which plays a significant role in immune clearance by bringing immune complexes from peripheral blood to macrophages in liver and spleen.^[90] It has been reported that the rate of clearance of immune complexes from the circulation is directly proportional to the number of CR1 molecules expressed on erythrocytes.^[90-92] CR1 can act as a negative controller of the complement cascade, mediates immune adherence and phagocytosis, and obstructs both the classic and alternative pathways.^[40,91]

Erythrocytes from different healthy people may show up to 10-fold variation in the number of CR1 molecules per cell

that may vary in the range of 50–1200 molecules per cell.^[90] Several single nucleotide polymorphisms have been found in the CR1 gene, and minimum three polymorphisms of CR1 gene are associated with erythrocyte surface density of CR1 molecules, which in turn related to the rate of immune complex clearance from the circulation. The most widely studied three single nucleotide polymorphisms of CR1 gene are A3650G (His1208Arg), intron 27 HindIII (T520C), and Pro1827Arg.^[90] The number and adhering activity of CR1 on erythrocyte in patients with GBC was reported to be lower as compared to patients with chronic cholecystitis, cholelithiasis, and healthy controls.^[40,91]

E-cadherins (CDH1)

E-CDH1 is one of the most important molecules in cell-cell adhesion in epithelial tissues. It is localized on the surfaces of epithelial cells in regions of cell-cell contact known as adherens junctions. E-CDH1 is located on chromosome 16q22.1, which is critical for the development and Ca²⁺ maintenance of dependent cell adhesion, polarity, and differentiation of epithelial cells. It has been found that the significant differences in the protein expression of E-CDH1 between normal, inflamed, and cancerous gallbladder tissues. Reduced expression of E-CDH1 was reported in 67% of GBC. It has been observed that loss of E-CDH1 expression was a more prominent event in GBC as compared to chronic cholecystitis, xanthogranulomatous cholecystitis, and normal GB. Many reports suggested that the expression of E-CDH1 diminishes as the tumor progresses.^[93,94]

ANGIOGENESIS FACTOR

In most cancerous condition, the development of malignant tumors is dependent on the genesis of new blood vessels (angiogenesis) to nourish malignant tumors.^[95]

Thrombospondin-1 (TSP-1)

TSP-1 is a trimeric 450 kDa glycoprotein of the ECM and consists of three identical disulfide-bonded chains that affect cell adhesion, motility, and growth. TSP-1 has been reported in platelets and it is secreted by platelets, fibroblast cells, smooth muscle cells, monocytes, macrophages, and numerous neoplastic cells.^[96-98] TSP has been involved in the regulation of cell growth, cell adhesion, proliferation, cell motility, cytoskeletal organization, inflammation and wound healing, and differentiation of different cell types.^[96-99] TSP-1 is supposed to be a significant regulator of tumor growth and lymph node metastasis. TSP-1 expression was lacking in normal cells, even T1 cancers, but expressed 74.5% in T2 and T3 GBC along with high expression of lymph node metastasis.^[40,96]

Cyclooxygenase-2 (COX-2)

COX enzyme is catalyzed conversion of free arachidonic acid to prostaglandins.^[73,100,101] Isoform of COX, COX-2, is induced by mitogen, cytokines, and growth factors which involved in regulation inflammation, cell growth, and tumor development.^[40,102] Over-expression of COX-2 was detected in GBC cases with high proportions in dysplasia and invasive carcinoma as compared with normal epithelium^[74,100] COX-2 expression reported to be reduced in adenocarcinoma (59.2%) when compared with dysplastic epithelium (70.3%).^[74,103] It has also been noted that the increased neovascularization induced by COX-2 might be responsible for the poor prognosis of human GBC patients.^[73]

VEGF-A

The VEGF-A is a potent proangiogenic agent involved in many types of carcinogenesis and an attractive target for cancer therapy. It has been reported that high expression of VEGF-A was observed in 81% of GBC and 5.1% of chronic cholecystitis. VEGF-A is highly expressed in GBC and correlates with poor prognosis, suggesting that VEGF-A expression might be used as a biomarker for predicting malignant behavior and for identifying a subset of patients.^[104,105]

CELL CYCLE REGULATORS

The cell cycle is regulated by several mechanisms that fortify correct cell division. Evading from normal cell cycle regulation is critical for the various processes of carcinogenesis. Cell cycle regulators more often altered in cancers are those governing G1-S-phase progression. G1-S transition is governed through interaction of various types of molecules, such as cyclin-dependent kinases (CDKs), their positive regulators, cyclins (D1, D2, D3, and E), and their negative regulators, CDK inhibitors, and Rb protein.^[106] Very less information is available about the cell cycle regulators which get altered in GBC.^[40]

Cyclin E

Cyclins are important proteins in cell-cycle regulation that is associated with and trigger different cyclin-dependent kinases at different stages of the cell cycle and allows them to pass through particular stage in the cycle.^[107] Cyclin E is a regulatory subunit of the cell cycle kinase CDK2 and plays an important role in normal cell proliferation and development through promotion of S phase of the mammalian cell cycle.^[108,109] Cyclin E seems to have the most significant role in many different types of tumor.^[107,108] Cyclin E was expressed in the adenocarcinoma of gallbladder tissue in 33.3% of patients, whereas adenoma expressed 12.5%^[40,98]

which suggests that higher expression of cyclin E in adenocarcinoma of the gallbladder is related to a higher rate of cell proliferation.^[107]

Cyclin D1

Restriction point present in cell-cycle progression, the restriction point of G1-S is very important. Cyclin D1 may promote the progression of the cell cycle to S by CDK4/CDK6-mediated phosphorylation of the Rb protein, which is, in turn, regulated by CDK inhibitors, such as p16INK4 protein.^[110] Cell-cycle drive is normally regulated by cyclins and cyclin inhibiting proteins.

Cyclin D1 plays role in cell cycle progression through two mechanisms: (a) Cyclin D1-CDK complexes deactivate Rb protein by phosphorylation; and (b) these complexes bind and sequester Cip/Kip proteins stoichiometrically.^[106] Dysregulation of cyclin D1 plays a critical role in various malignancies. The previous study reported that the abnormal cyclin D1 expression rates in GBC and adenoma were significantly higher than those in chronic cholecystitis.^[110] The expression rates of anomalous cyclin D1 were found in 68.3% and 57.1% GBC and gallbladder adenoma, respectively. These expression rates were significantly higher than those observed in chronic cholecystitis (7.1%)^[40,110] specimens with overexpression of cyclin D1 showed a higher frequency of lymphatic permeation, venous permeation, and lymph node metastasis.^[40,111]

Cyclin-dependent kinase inhibitor 1B (p27Kip1)

p27kip1 protein (p27), a cyclin-dependent kinase inhibitor, has an inhibitory effect on the G1-to-S phase transition in the cell cycle through its negative effects on cyclin E/CDK2 and cyclin A/CDK2. Lower expression of p27Kip1 is associated with poor prognosis in a variety of malignant diseases.^[112,113] In the case of GBC, lower expression was reported in 43–65% cases.^[93]

APOPTOSIS

Apoptosis was primarily described by its morphological characteristics, such as cell shrinkage, membrane blebbing, nuclear fragmentation, and chromatin condensation. Apoptosis is a gene-regulated program that has profound implications in developmental biology. Cell counts can be regulated by factors that affect cell survival as well as those that regulate proliferation and differentiation. Moreover, the reason for apoptosis implies that programmed cell death (PDC), i.e., apoptosis may be disrupted by a mutation in proapoptotic genes that show that defects in apoptotic genes are thought to be contributed to a number of human diseases, ranging from neurodegenerative disorders to malignancy.^[114]

Table 1: Summary of molecular genetic changes involved in gallbladder carcinoma.

Genes	Major normal function	Molecular genetic changes in GBC	Expression in GBC	References
Oncogene				
K- <i>ras</i>	GTPase activity and hydrolyzes guanosine triphosphate to guanosine diphosphate and are involved in signal transduction, molecular switches in the cell cycle	Point mutation	Higher (10–67%)	[24,31,40,47,93]
EGFR	Active form of EGFR stimulates intracellular pathways that promote cell proliferation and angiogenesis and also to evasion of apoptosis	Point mutation and overexpression	Higher (63.4%)	[40,47,93]
HER-2/ <i>neu</i> (ERBB2)	HER-2 is plasma membrane-bound cell surface receptor of the tyrosine kinase gene family and its protein clusters in cell membrane that play a role in tumorigenesis	Overexpression	Higher (16–64%)	[40,93]
Tumor suppressor gene				
<i>TP53</i>	<i>TP53</i> gene encodes a protein that binds to DNA, which in turn stimulates p21 that interacts with a cell cycle regulator (<i>cdk2</i>)	Over-expression, point, frameshift mutation	Higher (58.3–100%)	[40,93]
FHIT	Encodes a cytoplasmic protein with diadenosine triphosphate (Ap3A) with hydrolase activity, and it involves in regulation of DNA replication, and apoptosis	Downregulation, MSI, and LOH	Lower (45–75%)	[21,40,93]
Retinoblastoma	Dysfunctional of Rb results in unscheduled cell proliferation, apoptosis, and widespread developmental defects, leading to embryonic death	Downregulation, MSI, and LOH	Lower (58.5%)	[40,93]
Adhesion molecules				
MUC1	Play a role in cell to cell interactions, signaling, and involvement in metastasis	Higher expression	Higher (78%)	[40,93]
Erythrocyte CRI	CRI can act as a negative regulator involved in the complement cascade, which mediates immune adherence and phagocytosis through inhibiting both the classical and alternative pathways	Low activity	Lower	[40,93]
Cadherins	Tissue invasion (play an important role in cell-cell adhesion)	Overexpression of N-cadherin, P-cadherin and lower expression of E-cadherin	Higher expression of N-cadherin (55%), P-cadherin (53%), and loss of E-cadherin expression (67%)	[21,93,94]
Angiogenesis factor				
TSPI	Involved in the regulation of cell growth, adhesion, proliferation, motility, cytoskeletal organization, inflammation and wound healing, and differentiation of various cell types	Higher expression	Higher (74.5%)	[93,96]
COX-2	Catalyzed conversion of free arachidonic acid to prostaglandins and involved in the regulation of inflammation, cell growth, and tumor development	Overexpression	Higher (59.2–71.9%)	[73,93]
VEGF-A	The VEGF-A is a vigorous proangiogenic agent involved in the carcinogenesis	Overexpression	Higher (81%)	[93,104]
Cell cycle regulators				
Cyclin E	Involved in the regulation of transition from the G1 phase to the S phase of cell cycle	Overexpression	Higher (33%)	[40,93]
Cyclin D1	Regulate the G1 phase of the cell cycle	Overexpression	Higher (41–68.3%)	[40,93]

(Contd...)

Table 1: (Continued)

Genes	Major normal function	Molecular genetic changes in GBC	Expression in GBC	References
p27Kip1	It is an important cell-cycle regulatory protein that interacts with cyclin CDK2 and CDK4, inhibiting cell cycle progression at G1. Its degradation is caused by its CDK dependent phosphorylation, and successive ubiquitination by SCF complexes is required for the cellular transition from quiescence to the proliferative state	Low expression	Lower (43–65%)	[93,113]
Apoptosis	Involved in apoptosis and inflammation Play an important role in neoplasia by inhibiting tumor cell apoptosis	Overexpression Overexpression	Higher (77–95%) Higher (34.7%)	[40,93] [40,93]
Survivin	Survivin is a class of the IAP family which inhibit caspase activation and lead to negative regulation of apoptosis or programmed cell death	Overexpression	Higher (67.5%)	[124,125]

GBC: Gallbladder carcinoma, EGFR: Epidermal growth factor receptor, FHTT: Fragile histidine triad, MSI: Microsatellite instability, LOH: Loss of heterozygosity, CRI: Complement receptor 1, TSP1: Thrombospondin-1, COX-2: Cyclooxygenase-2, VEGF-A: Vascular endothelial growth factor A, CDK: Cyclin-dependent kinase, IAP: Inhibitor of apoptosis

Caspases (CASP)

Studies have shown that the alteration in apoptotic pathway genes can cause defective apoptosis.^[115] CASPs are very conserved intracellular aspartate-specific cysteine proteolytic enzymes, which are capable to cleave proteins with a consensus sequence at as tartaric acid alanine residues and play significant intermediaries of the apoptotic pathway.^[116-118] Fourteen different CASPs have been reported in mammalian cells.^[116]

CASP-8 is an important apoptosis regulatory gene which was assessed in cancer studies. They suggest that low penetrance variants in CASP-8 gene may affect GBC vulnerability.^[115,116] Furthermore, many studies have been shown that active CASP-3 is required to induce apoptosis in response to chemotherapeutic treatments using treatments of taxanes, 5-fluorouracil, and doxorubicin.^[119]

CASP-3 is a 32-kDa pro-enzyme, which was cleaved into 12 and 17-kDa subunits. Two 12-kDa and two 17-kDa subunits of the CASP-3 subunits were re-associated to form the functionally active CASP-3 enzyme. The initiator CASPs (CASP 8, 9, and 10) are activated by apoptotic stimulation factor, which further activates effector CASPs.^[120] After activation of the effector CASP molecules which include CASP-3, 6, and 7 that initiates the cleavage of many important cellular proteins which include poly (ADP-ribose) polymerase, gelsolin, inhibitors of CASP-activated DNase, 4-GDI, α - and β -fodrin, and EGFRs.^[118-120] CASPs caused blebbing of the membrane, nuclear destruction condensation of chromatin, and DNA fragmentations.^[117-119] CASP-3 plays crucial role in apoptosis so that we evaluated the expression levels of procaspase-3 and activated CASP-3 in GB tumor tissue samples.^[119] The level of apoptosis was higher in Grade II-III as compared with Grade I tumors or epithelial dysplasia, and also, tumors invading beyond serosa or into other organs (TI-T4) had a higher apoptotic index than other tumors (average apoptotic index was $0.68 \pm 0.91\%$).^[117] CASP 3 expression reported in 95% of GBC cases. CASP 6 and 8 expression was also observed, each in 77% GBCs.^[40,117]

Bcl-2

Bcl-2 is an intracellular membrane protein capable of obstructing the PDC prompted by various stimuli. The Bcl-2 gene is an anti-apoptotic membrane-associated molecule that exists in the nuclear envelope and outer membrane of mitochondria.^[121] The Bcl-2 family proteins are significant regulatory components of the intracellular apoptotic pathway which is an important death regulator of mitochondrial integrity and mitochondria-regulated apoptosis.^[121,122] Bcl-2 protein is supposed to play a role in cancer development by inhibiting tumor cell apoptosis.^[122,123] Bcl-2 was reported over-expressed in 34.7% of the gallbladder cancer.^[40,122]

Survivin

Survivin is the most effective member of human inhibitor of apoptosis. Survivin expressed in the G2/M phase of the cell cycle that plays a role in cell division, cell survival, and proliferation. Survivin overexpression is reported during fetal development but low in healthy adult tissues. Overexpression of survivin has been studied in various cancers. Survivin expression was significantly overexpressed in GBC than cholelithiasis. The results from different studies suggested its role in gallbladder carcinogenesis.^[124,125]

OTHER GENES

Hedgehog pathway genes

Hedgehog signaling promotes tumor growth and it might take part in the proliferation of tumor stem cells.^[79] The hedgehog pathway is an emerging therapeutic target for several cancers, including GBC. However, only one study has been reported so far.^[126] Novel sonic hedgehog mutations were found 10 GBC patients.^[126] Thus, further research is required to unravel the diagnostic role of hedgehog pathway genes.

Wnt signaling pathway genes

Yadav *et al.* reported genetic variants in Wnt signaling pathway which was found to be significantly associated with increased risk and poor survival. The study also depicted that AXIN-2 and APC play an important role in the regulation of the stability of β -catenin in the Wnt signaling pathway. Thus, the deregulation of β -catenin is an important event in the genesis of gallbladder carcinogenesis. Genetic variants of GLI-1, AXIN-2, and APC genes consistently showed association across all analyses, suggesting genetic variants are associated with advanced GBC susceptibility and treatment outcomes.^[127]

Methylene tetrahydrofolate reductase (MTHFR) gene

The MTHFR gene is essential for DNA methylation and synthesis. Polymorphisms in the MTHFR gene have been studied as possible risk factors for a variety of diseases including cancer. A recent study concluded that increased frequency of the A1298C MTHFR gene polymorphism was higher in GBC in comparison to cholelithiasis and normal control and associated with gallbladder carcinogenesis.^[128]

Non-coding RNAs (ncRNAs)

The transcriptome studies and genome tiling arrays specify that more than 90% of the human genome transcribes

ncRNAs, while only 2% is thought to be transcribed and translated into proteins (Khandelwal *et al.*, 2015). ncRNAs can be classified into two groups based on their size: (i) One group includes short RNAs <200 nucleotides (nt) in length, such as micro RNAs (generally between 22 and 25 nt), snoRNAs, and piwiRNAs (between 26 and 31 nt), and (ii) the other group includes long ncRNAs (lncRNAs) of around 200 nt or more.^[129-131]

MicroRNAs (miRNAs)

miRNAs are small (18–25 nt), noncoding, and single-stranded RNA molecules that regulate gene expression at post-transcriptional and translational levels.^[132] Nearly, 30% of the global gene expression is probably regulated by miRNAs. Aberrant expression of multiple miRNAs has been reported to be associated with many human cancers, and >50% of miRNA genes are found to be present with a satisfactory response to chemotherapy located in cancer-related chromosomal regions.^[133] Numerous studies have reported aberrant miRNA expression in gallbladder cancer tissues and suggested specific miRNAs that are functionally involved in gallbladder cancer development.^[134,135]

lncRNAs

lncRNAs, normally found as endogenous cellular RNAs, lack the Kozak consensus sequence and open reading frame of significant lengths. Although few studies have shown their ability to encode proteins that means these bi-functional RNAs can have both protein-coding and coding-independent functions.^[130,136]

Initially thought to represent transcriptional noise in early RNA-Seq studies, lncRNAs are now regarded as functional RNA elements, generally expressed at low levels in a tissue-specific and time-restricted manner, with half-lives varying from <2 h to more than 16 h, and a median half-life of ~3.5 h. lncRNAs play significant roles in many biological processes, such as transcription, splicing, translation, protein localization, epigenetic gene expression, cell structure integrity, cell cycle, heat shock response, imprinting, stem cell pluripotency and reprogramming, embryogenesis, and regulation of the immune response. Because of their roles in the regulation of multiple molecular pathways associated with changes in gene expression, dysregulation of lncRNA associated with several types of cancer. Enforced expression of some cancer-promoting lncRNAs can enhance the proliferation and migration in cancer cells through regulating cell cycle protein and promoting epithelial–mesenchymal transition process. However, overexpression of some anticancer lncRNAs can inhibit the development of cancer, including proliferation inhibition, migration, suppression, and apoptosis induction.^[129,137]

Some studies demonstrated that aberrantly expressed lncRNAs may serve a role in the occurrence and development of GBC. Although numerous lncRNAs have been discovered over the previous decade, the biological functions of lncRNAs in GBC and their underlying molecular mechanisms remain unclear.^[138]

CONCLUSION

GBC is a highly lethal disease with wide geographical incidence. Despite the efforts of many investigators, GBC continues to represent a major challenge in oncology. Studies have shown variety of genes to be modified in GBC, but there is lack of sufficient knowledge about the exact role of these modifications in GBC. The present review article provided a summary of the molecular genetic changes occurred in GBC. This will be helpful for better understanding of gallbladder carcinogenesis. Further, exploration of complete genetic profile may provide a complete picture of the disease progression which will open new options for developing diagnostic biomarkers and targeted therapy in GBCs.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

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Conflicts of interest

There are no conflicts of interest.

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