REVIEW ARTICLE
GENETIC RISK FACTORS ASSOCIATED WITH
GALLBLADDER CARCINOMA
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Gallbladder cancer (GBC) is an aggressive biliary tract cancer with wide geographic diversity. Various genetic modifications of GBC, including mutations in p53, KRAS, p16 and retinoblastoma (RB) gene. Mutations in p53 lead to carcinoma, while point mutations in KRAS leads to hyperplasia. KRAS mutations are often found in both pancreatico-biliary ducts junction and in neoplastic loci in gallbladder polyps. The survival rate for overall 5-year of GBC patient is less than 1%. GBC is crucial for diagnostic and prognostic markers and potential drug targets. Ovid-MEDLINE, PubMed, CINHAL and Google Scholar databases were searched using keywords gallbladder carcinoma, neoplasia, tumour, tumor, adenocarcinoma, biliary tract carcinoma, gene mutations, KRAS, p53, RB, and p16. Ten out of 470 research articles were finally included. It was observed that loss of heterogeneity and mutations of KRAS, p53, p16 and RB involve in the disruption of cell cycle leading to continuous cell division and cancer.

Keywords: Gallbladder carcinoma, genetic mutations, KRAS, p53, p16, retinoblastoma, RB

INTRODUCTION AND BACKGROUND

Gallbladder carcinoma (GBC) is a lethal malignancy with a range of latitude and gender inequalities, and 5-year survival for GBC is <1%.1 The commonest symptoms of GBC are vomiting, abdominal pain and jaundice. Complete resection of the gallbladder is sometimes the only cure for the disease. However, because of a huge disparity in signs and symptoms, the disease usually spreads to other organs, such as the liver, at the time of diagnosis and is beyond any intervention.2 Even in patients in whom surgical resection is feasible, the anatomical complexity of the portal hepatic system as well as the morbidity and mortality associated with resection and the risk of tumour metastasis are secondary to manipulation deterrents. Recurrence rates are also high in postoperative resections.3

With the advent of genomics, a mounting understanding of the molecular basis of different tumours is under way. However, studies in GBC are still limited. Early diagnosis of the disease is not possible and conceivable despite advances in imaging modalities.4 To date, no conclusive biomarker has been identified for diagnosis or prognosis of GBC, as recognition is restricted by the availability of biological material for profiling. However, a variety of genetic changes are recognized to be involved in gallbladder cancer.5 In this review, we focused on the most frequently reported mutations in p53, KRAS, p16, retinoblastoma (RB), or protein (pRB) gene modifications and their clinical implications.

Gallbladder carcinogenesis

Studies have identified three different pathways of pathogenesis in GBC; these are (i) de novo growth, (ii) carcinoma adenoma formation, (iii) progression of hyperplastic carcinoma associated with abnormal pancreatic-biliary duct transition (AJP).6

The mechanism of GBC occurs via two main pathways. These two paths are: one is the carcinoma sequence through dysplasia and the second is the carcinoma sequence of the adenoma formation.

Dysplasia-carcinoma sequence

In the carcinoma sequence of dysplasia, normal wild type cells are mutated by changes in normal cell genes that convert proto-oncogenes into oncogenes. This results in a step-by-step progression from normal GBC to invasive carcinoma over a period of several years. The RAS family (HRAS, NRAS and KRAS) of the proto-oncogene is often mutated. The most notorious gene mutation on codon 12 in the KRAS is generally associated with human GBC formation.7 This review involved the collection as well as selection of comprehensive review data from past 30 years about gall bladder carcinoma and data analysis (Figure-1).

Genetic mutations
KRAS

As already described the KRAS is a member of the RAS gene family with HRAS and NRAS.7 Its role in the development of human carcinoma conditions has widely been accepted in the field of human oncology. KRAS, in particular, is considered to be one of the most frequently-affected genes in a variety of tumours8, with mutations leading to KRAS wild-type amplification and triggering oncogenic conversions in the gallbladder; ovary, stomach, uterus, lung and colorectal carcinomas.9 Table-1 shows the collective information of previous studies done on different mutation related with GBC.
RESULTS AND DISCUSSION

In GBC is reported that the incidence of KRAS mutation at codon 12 in exon 2 (GGT> GAT) ranges from zero to 80 percent.\textsuperscript{10} This wide range can be attributed to insensitivity of use or to ethnic and geographical differences in the populations studied.\textsuperscript{11} Also it is observed that the incidence of codon 12 mutation is significantly higher in GBC where there is a synchronous state of anomalous junction of pancreatico-biliary duct (AJPBD) than in other types.\textsuperscript{12,13} This suggests that KRAS mutation detection is a useful molecular diagnostic marker for the early stage of carcinogenesis in the gallbladder with AJPBD.\textsuperscript{14} A new KRAS polymorphism is detected in codon 25 (CAG>CAT; Gln25His) in exon 1. This is present both in the germ line as a GBC woven tissue and is therefore, a known pathogenic variant.\textsuperscript{15} Research in patients with AJBPD for this polymorphism may facilitate early diagnosis of GBC.\textsuperscript{16–22} However, other mutations are also identified in GBC with KRAS predominantly in South Asian populations.\textsuperscript{23} KRAS mutations are not observed in the cases of adenomas.\textsuperscript{24,25}

The normal protein product pathway of the KRAS gene is through the GTPase pathway that is involved in cell signalling mechanisms. Subsequent to mutation, the KRAS mutant results in the activation of mitogen-activated protein kinase (MAPK), which progresses the cell cycle through cell proliferation, cell survival, invasion, and metastasis.\textsuperscript{26}
Interestingly, the RASSF1A gene on chromosome 3p21, which has also been recently classified as a tumour suppressor gene, shows to have a positive association with the recurrent allelic losses in GBC.\textsuperscript{27} The RASSF1A protein has a strong homology with the Nore1 mouse RAS protein which likewise acts via a GDP-dependent method and is required for receptor activation.\textsuperscript{6,28,29}

**Adenoma-carcinoma sequence**

The carcinoma sequence through the adenoma formation includes mutations in tumour suppressor genes (TSGs) with loss of heterogeneity (LOH) on polymorphic loci flanking TSG.\textsuperscript{30} This have been documented as features of cancer formation. In GBC, LOH often occurs in a large number of chromosome sections, including 1p, 3p, 5q, 8p, 9p, 9q, 13q, 16p, 16q and 17p.\textsuperscript{31} We are interested here in the most frequent genetic changes in GBC, particularly in p53 at the 17p13 locus\textsuperscript{32} and in the retinoblastoma (RB) and p16 genes.\textsuperscript{33}

**p53**

The most frequently transformed tumour suppressor gene p53 is located on chromosome 17p13\textsuperscript{34} and leads to gallbladder dysplasia. Immunohistochemistry (IHC) shows over expression of p53 in the initial phase of GBC.\textsuperscript{35} A significant feature of p53 is its predisposition to remain dormant in normal gallbladder cells. Unlike the normal protein product or p53, the aberrant p53 protein is likely to accumulate in cell nuclei and can be detected by mutations in the IHC gene.\textsuperscript{34} p53 leading to LOHs noted in more than 50% of the GBC cases.\textsuperscript{37} p53 mutations are similarly observed in de novo carcinoma regardless of size and depth of invasion, and in GBC with AJBPD, but have not been documented in adenocarcinomas.\textsuperscript{35} Studies to date have focused on mutations in exons 5 to 8.\textsuperscript{18} Point mutations result in alterations in phosphorylation in mutated p53, results in altered β-catenin characteristics or cell adhesion in GBCs causing metastasis and invasion of cancer cells.\textsuperscript{19}

**Retinoblastoma (RB)**

Mutations in the tumour suppressor RB gene are associated with GBC formation 17p13\textsuperscript{35} and identified as a prognostic factor for the diseased condition.\textsuperscript{36} The changes in this gene on chromosome 13q have been documented in the consequence of GBC formation. The detection of this modification is done by the deletion of the RB locus on chromosome 13, which leads to the abnormal formation of the RB1 Protein (PRB1). The abnormal generation of the abnormal retinoblastoma protein results in its localization in the nucleus altering the translation of enzymes production for DNA synthesis and therefore cell replication and proliferation.\textsuperscript{21} The complete process occurs by PRB1 binding to the elongation factors (E2F), while phosphorylation of PRB1 allows the release of E2F driving the cell to the S phase of the cell cycle. This process of cell cycle is known to be driven by cyclin D, in combination with CDK4 and CDK6, and cyclin E in combination with CDK2.\textsuperscript{22} GBC linked to AJBPD is known to have the highest allelic loss and the same is correspondingly observed with RB gene mutation. The loss of functional pRB is also related to p16 mutations which may induce the dysregulation of cell cycle and malignancies. This is conversed far ahead in the review for better understanding about p16 mutations.

**p16**

The loss of manifestation of the protein in tumour suppressor p16 gene also known to be the cyclin-dependent kinase inhibitor encoded by p16 gene and SMAD4/DPC4 can lead to the formation of non-small cell lung carcinoma, gallbladder or biliary tract non-small cell carcinoma (NSGBC).\textsuperscript{20} The frequency of SMAD4/DPC4 loss is higher with peri-hilar and proximal-hepatic tumours of the biliary tract. The loss of the SMAD4/DPC4 protein also causes GBC in situ, suggesting that inactivation may occur in early GBC lesions.\textsuperscript{23} The appearance of SMAD4/DPC4-protein loss in these tumours may indicate the loss of heterozygosity on chromosome 18q, previously identified in allele typing studies of gallbladder carcinoma. The tumour suppressor genes that are involved in gallbladder carcinogenesis are in 5-59% of subjects with established AJBPD\textsuperscript{35} as p16 normally plays a vital role in cell cycle control, the cyclin-dependent kinase inhibitor, it inhibits the production of cells from G1 phase to S phase.\textsuperscript{32}

**Clinical implications**

There is little research on GBC-related genetic mutations known to be the third most common digestive cancer in the South Asian sub-continent, in which Pakistan is also located.\textsuperscript{16} The only curative intervention in GBC is complete surgical resection of the gall bladder. However, this only offers benefits for people with localized disease. Limited treatment options are available for patients,\textsuperscript{39} with progressive and non resectable gallbladder cancer due to a shortage of identified molecular targets. In recent years, research has focused on the molecular mechanisms of disease in order to identify treatment goals, in addition to diagnosis and prognosis. The importance of these genetic mutations in KRAS, p53, p27, p16 and pRB may help clinicians and surgeons identify them as GBC markers for detection and prognosis, as well as potential therapeutic targets for the treatment and healing. It is useful to recognize the importance of KRAS and p53, because it is an important potential are presented as independent prognostic markers, and may also be useful for identifying early stages of lesions that may develop in cancers malignant. In addition, it is important to
identify new therapeutic agents which contain antiproliferative effects in cancer cells. 17

Molecular markers of cancer thus identified will be useful not only for cancer recognition and predictive effects, but correspondingly for the construction of the management program for patients with GBC. Previous studies with the above said genetic alteration are known to have shown a high potential to become autonomous predictive markers of GBC. Therefore, their role in the efficacy of therapeutic targets for the treatment of the disease is absolutely essential. They can also be useful for identifying precancerous lesions that can lead to malignant cancers, particularly in the case of p53. GBCs have also repeatedly shown p53 gene mutations as well as KRAS, and RB gene mutation. Consequently, these mutations may also be useful in understanding the mechanism and extension as well as prolongation of the GBC pathogenesis.

CONCLUSIONS

This review focuses on the common genetic changes that occur in GBC. LOH and mutations affecting KRAS, p53, p16, and RB play a role in the progression of GBC. The accumulation of these genetic changes results in disruption of the normal cell cycle leading to continuous cell division and cancer. Limited studies on genetic mutations have been reported in South Asian GBC patients. The absence of this literature in this review underscores the need for detailed studies in larger cohorts to identify new molecular targets that can be used for GBC diagnosis, treatment and prognosis, particularly in the Asian population where the disease is common.

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