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Original Research Article

Emerging need of molecular profiling in hepatobiliary cancer

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ABSTRACT

Background: Gallbladder cancer is a rare malignancy but represents almost 50% of all biliary tract cancer. Biliary cancers are highly fatal malignancies with a 5-year survival rate of approximately 20%. The prognosis of gallbladder cancer is poor due to the aggressive tumor biology, late presentation, complicated anatomic position, and advanced stage at diagnosis. Locally advanced and metastatic disease treatment is with palliative chemotherapy. Alarming sign of gallbladder cancer is overall decreased in incidence in older patients but increased in the younger population. So many mutations have been reported for the gall bladder cancer till date.

Materials and Methods: A prospective observational study was conducted over a period of 1 year at Asian Institute of Medical Sciences Faridabad which includes hepatobiliary carcinoma patients who are at stage III and stage IV of cancer. After getting the consent formalin fixed paraffin embedded biopsy samples, and 5 ml serum sample was collected in serum separator tube (SST). A whole genome sequencing was performed using Illumina HiSEQ, Illumina (NGS) technology, allows for high-throughput sequencing of DNA and RNA. Illumina's NGS is based on "sequencing by Synthesis" to detect the mutations.

Results: Most common mutation found was in the P53 gene. TP53 (p.Arg175His), TP53 (p.Arg306Ter), TP53 (p.Cys238Tyr), TP53 (p.Leu43Ter), TP53 (p.Glu339Ter), TP53 (p.Pro190Leu).

Conclusion: Mutations in the TP53 gene are a common feature of carcinoma of the gallbladder, and are associated with a more aggressive tumor phenotype, resistance to chemotherapy, and poorer overall survival.

1. Introduction

Carcinoma of the gallbladder is a highly aggressive and lethal malignancy, with a poor prognosis and a high mortality rate. It is one of the most common malignancies of the biliary tract, and its incidence has been increasing globally over the past few decades. The gallbladder is a small, pear-shaped organ located under the liver that stores bile, which is produced by the liver to aid in the digestion of fats. Carcinoma of the gallbladder can arise from the epithelial cells lining the gallbladder, and it is typically classified as adenocarcinom.[1] Globally, carcinoma of the gallbladder is a relatively rare malignancy, accounting for approximately 3% of all gastrointestinal tract cancers. However, its incidence is higher in certain regions, particularly in South America and Asia, where it is one of the most common malignancies of the digestive system. The highest incidence rates are observed in certain populations of Native Americans, such as the Pima Indians of Arizona and the Mapuche Indians of Chile.[2] Recent advances in genomic technologies have led to the identification of several new mutations that may play a role in the development of carcinoma of the gallbladder. For example, mutations in the TP53 tumor suppressor gene have been

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identified in a significant proportion of cases, suggesting that alterations in this gene may be an important driver of tumor genesis in this disease.[3] Additionally, mutations in the KRAS oncogene and the PIK3CA gene have also been reported in some cases, providing further evidence for the importance of these pathways in the development of this malignancy. Overall, the increasing incidence of carcinoma of the gallbladder and the identification of new mutations that may contribute to its development highlight the need for further research into the molecular mechanisms underlying this disease. This may ultimately lead to the development of new diagnostic and therapeutic strategies that can improve outcomes for patients with this aggressive malignancy.[4] Small molecules that can either protect p53 from its negative regulators or restore the functionality of mutant p53 proteins are gaining interest, and drugs tailored to specific types of p53 mutants are emerging. In parallel, there is renewed interest in gene therapy strategies and p53-based immunotherapy approaches.[5]

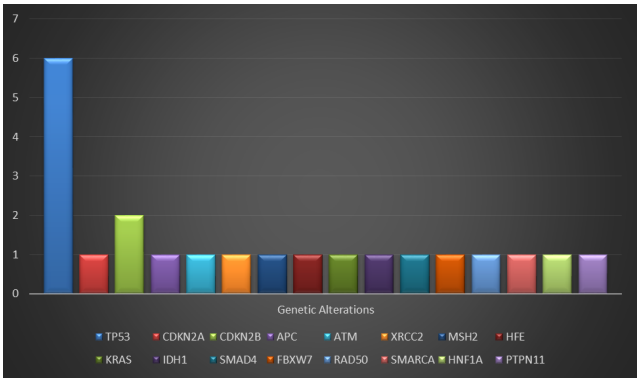

Figure 1: The TP53 gene was found mutated in most of the cases and the mutations were TP53 (p.Arg175His), TP53 (p.Arg306Ter), TP53 (p.Cys238Tyr), TP53 (p.Leu43Ter), TP53 (p.Glu339Ter), TP53 (p.Pro190Leu). The APC gene was found mutated at APC (p.Gly351Ter). The deletion and genetic alteration both found for CDKN2A. The deletion of CDKN2B and Amplification of FOXA1 gene was detected. No Translocation mutation was found in our study group

2. Materials and Methods

A prospective observational study was conducted over a period of 1 year at Asian Institute of Medical Sciences Faridabad which includes hepatobiliary carcinoma patients who are at stage III and stage IV of cancer with or without metastasis. After getting the consent formalin fixed paraffin embedded biopsy samples, and 5 ml serum sample was collected in serum separator tube (SST). A whole genome sequencing was performed using Illumina HiSEQ, Illumina (NGS) technology, allows for high-throughput sequencing of DNA and RNA. Illumina's NGS is based on "sequencing by Synthesis", which involves amplifying DNA or RNA fragments and then sequencing of them using fluorescently labeled nucleotides. We utilize this to find out the number of genetic mutations present among the samples.

3. Result

The genetic alterations which were found during the analysis are tabulated in the Table 1.

4. Discussion

Carcinoma of the gallbladder is a highly aggressive malignancy with a poor prognosis and a high mortality rate. Recent studies have identified new mutations that may play a role in the development of this disease, including mutations in the TP53 tumor suppressor gene, and are in accordance with our study. TP53 is a critical gene that plays a crucial role in regulating cell growth and division. Mutations in this gene have been implicated in the development of many different types of cancer, including carcinoma of the gallbladder. TP53 mutations were identified in approximately 45% of gallbladder carcinoma cases, making it one of the most commonly mutated genes in this malignancy. The same study also found that TP53 mutations were associated with a more aggressive tumor phenotype and a worse overall prognosis.[6] Another study conducted by N Sturm et al. (2022) demonstrated that TP53 mutations were associated with increased resistance to chemotherapy in patients with gallbladder carcinoma.[7] The authors of this study suggested that this may be due to the role of TP53 in regulating apoptosis, or programmed cell death. In the absence of functional TP53, cancer cells may be more resistant to chemotherapy-induced apoptosis, leading to poorer treatment outcomes. Another study by G Ishak et al. (2015) used single-cell sequencing to identify subclonal TP53 mutations in gallbladder carcinoma samples. The authors found that these mutations were associated with an increased risk of disease recurrence and poorer overall survival, highlighting the importance of accurate characterization of TP53 mutations for predicting treatment outcomes.[8,9] A similar study on cancer genome sequencing of 69 genes with recurrent genetic alterations reported in HCC. Unsupervised hierarchical clustering classified nonviral HCCs into three molecular classes (Class I, II, III), which stratified patient prognosis. Class I, with the poorest prognosis, was associated with TP53 mutations, whereas class III, with the best prognosis, was associated with cadherin-associated protein beta 1 (CTNNB1) mutations.[10] In an animal study model it has been Consistently, lnc-Ip53 is upregulated in multiple cancer types, including hepatocellular carcinoma (HCC). High levels of lnc-Ip53 is associated with low levels of acetylated p53 in human HCC and mouse xenografts, and is also correlated with poor survival of HCC patients.[11]

Table 1: Genetic alterations found in the hepatobiliary carcinoma

Age & gender	Diagnosis	Stage/ Met	Sample Type	Test	Treatment given	Genomic_ Alterations_ found	Amplification	Deletion	Translocation	MSI
56 M	Carcinoma Gall Bladder	Stage 4, Mets-lung	FFPE (Formalin Fixed Paraffin Embedded)	PET CT, MRI CT Scan	Chemotherapy	APC (p.Gly351Ter), TP53 (p.Arg175His), PTPN11 (p.Thr468Met), HNF1A (c.1309+1G>A), SMARCA4 (p.Glu763Ter), RAD50 (c.3752+1G>A), FBXW7 (p.Arg479Leu)	_	CDKN2A, CDKN2B	_	Stable
48 F	Carcinoma Gall Bladder	Stage 3	Plasma/serum	PET CT, CT Scan	Chemotherapy	_	_	_	_	Not mentioned
55 F	Carcinoma Gallbladder	Stage 3	FFPE	PET-CT, CT Scan	Chemotherapy, Radiotherapy, Surgery	TP53 (p.Arg306Ter), SMAD4 (p.Ser138Ter)	_	_	_	Stable
56 M	Ca Gall Bladder	Stage4-liver, lung	FFPE	PET-CT, CT Scan	Chemotherapy, surgery, radiotherapy	TP53 (p.Cys238Tyr)	_	_	_	Stable
59 F	Gall Bladder, Liver, Extrahepatic Bile Duct Cancer	Metastatic-visceral peritoneum, lymphovascular, perineural	FFPE	PET-CT, CT Scan	Chemotherapy, surgery, radiotherapy	CDKN2A (p.Ala68Val)	_	_	_	Stable
65 M	Gall Bladder Carcinoma	Metastasis - lung and liver	FFPE	PET-CT, CT Scan	Surgery	IDH1 (p.Arg132Cys), KRAS (p.Gly12Val)	_	_	_	Stable

Continued on next page

Table 1 continued

48	F	Carcinoma Gall Bladder	Stage 3	FFPE	PET-CT, CT Scan	CTNNB1 (Tegavivint), TP53 (Olaparib (AZD2281)), XRCC2 (LY2606368 (Prexasertib))	CTNNB1 (p.Ser45Phe), HFE (p.His63Asp), TP53 (p.Leu43Ter), XRCC2 (p.Leu117fs)	_	_	_	Stable
50	M	Metastatic Carcinoma Gall Bladder	Stage 3	FFPE	PET-CT, CT Scan	TP53: Olaparib (AZD2281) Alone and in Combination with AZD1775, AZD5363 or AZD673;APR-246 in combination with Pembrolizumab	TP53 (p.Glu339Ter)	FOXA1 (Amplification)	_	_	Stable
49	F	Carcinoma Gall Bladder	Stage 3	FFPE	PET-CT, CT Scan	Surgery	ATM (c.1802+1del), MSH2 (p.Gln429Ter), TP53 (p.Pro190Leu)	_	_	_	Stable

Further multicentric and with larger sample size research is needed to better understand the molecular mechanisms underlying TP53-mediated tumorigenesis in this disease, which may ultimately lead to the development of new diagnostic and therapeutic strategies for improving outcomes in patients with this aggressive malignancy. Along side early diagnosis is very important because early treatment is also implemented and prognosis of the disease improved. A similar study reported Five subtypes were revealed in primary liver cancers. Patients featured terminally exhausted immune characteristics showed worse outcome. Increased intratumor heterogeneity, enriched somatic TP53, KRAS, APC, and PIK3CA mutations and hyperactivated hypoxia signaling accounted for the formation of vicious subtypes.[12] A similar result also demonstrated in a study, where they performed an exome sequencing of 45 driver genes in 100 paired samples from HCC patients including tumors and matched adjacent normal tissues using Illumina HiSEQ 2000 platform and the most frequent mutations were: TP53 (20%), RET (6%), PLCE1 (5%), PTEN (4%) and VEGFR2 (3%).[13] Patients with mutations in TP53 had a lower overall survival (OS) (P=0.002) than those without mutations these findings are in accordance with our study. Liu ZH et al. also reported TP53 and CTNNB1 were identified as exhibiting mutations in hepatocellular cholangiocarcinoma. ARID1A, PBRM1, and IDH1 were frequently mutated in ICC. RYR3, FBN2, and KCNN3 are associated with cell migration and metastasis and might be driver genes in hepatocellular cholangiocarcinoma.[14] TP53 and LAMA3 existed relative higher mutation frequency in HCC, and expressed higher in tumor tissues.[15] These findings are in accordance with our study.

5. Conclusion

We concluded that the risk of hepatobiliary carcinoma increases with the increase in the genetic alterations. The P53 gene is most commonly involved gene for the carcinogenic transformation. mutations in the TP53 gene are a common feature of carcinoma of the gallbladder, and are associated with a more aggressive tumor phenotype, resistance to chemotherapy, and poorer overall survival.

6. Limitation of Study

The study only comments on the frequency of the CA gallbladder mutations found in patients. The pathological aspects of mutation were not detailed in the study.

7. Source of Funding

None.

8. Conflict of Interest

None.

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