Egyptian Journal of Medical Microbiology Volume 34 / No.2 / April 2025 159-167 Online ISSN: 2537-0979

ORIGINAL ARTICLE

Genetic Variants of EGFR and EpCAM in association with HCC Susceptibility in HBV Infected Iraqi Patients

[1]Khalid R. Majeed*, [2]Wiaam A. AL-Amili, [3]Safaa A. A. Al-Waysi

[1]Department of Medical Laboratory Technique, Al-Nasiriyah Technical Institute, Southern Technical University, Thi-Qar, Iraq

[2]Department Genetic Engineering/ Institute of Genetic Engineering and Biotechnology for Postgraduate Studies / University of Baghdad / Baghdad / Iraq

[3]Medical City, Hepatology and Gastroenterology Teaching Hospital

Key words:
HCC, HBV, EGFR, EpCAM. PCR-HRM

Corresponding Author:
Khalid R Majeed, PhD, Department of Medical Laboratory Technique, Al-Nasiriyah Technical Institute, Southern Technical University, Thi-Qar, Iraq
khalid.gen12@stu.edu.iq

ABSTRACT

Background: *Primary liver cancer (PLC) is a highly lethal malignancy and the third most common cause of tumor-associated death annually. Hepatocellular carcinoma (HCC) accounts for 75–85% of all PLC cases. Hepatitis B virus (HBV) remains a major risk factor for HCC. HBV share important functions in the (EGFR) signaling pathway, with EpCAM potentially serving as a cancer stem cell marker in HCC.* ***Objective:*** *to assess whether EGFR and EpCAM gene variants in HBV patients in Iraq contribute to the development of HCC.* ***Methodology:*** *A total of 120 samples were collected, comprising 40 HCC patients (group I), 40 chronic HBV (CHB) patients (group II), and 40 healthy controls (group III). SNPs identification was done using the polymerase chain reaction-HRM (PCR-HRM) curve analysis.* ***Results*** *Genotyping rs884225 (EGFR gene), both genotype and allele frequencies show no statistically significant differences between HCC, CHB patients, and HCs (all p-values > 0.05). In contrast, rs62139665 in the EpCAM gene showed significant associations. The AA genotype and A allele were strongly linked to HCC ($p < 0.01$) and moderately to CHB ($p < 0.05$). The AA genotype demonstrated a very high odds ratio for HCC (OR = 10.7), while the A allele showed a moderate association with CHB (OR = 1.8).* ***Conclusion:*** *The AA genotype and the A allele of rs62139665 in the EpCAM gene are potential genetic markers for susceptibility to HCC and CHB, whereas rs884225 in the EGFR gene does not significantly contribute in the risk for these diseases in Iraqi population*

INTRODUCTION

Primary liver cancer (PLC) is a high-risk malignant tumor, with an estimated 906,000 new cases identified and 830,000 deaths, or 4.7% of all cancer cases in 2020. It ranks third in cancer-related deaths and sixth in the overall cancer incidence. Uncommon forms of primary liver cancer are intrahepatic cholangiocarcinoma (ICC) and hepatocellular carcinoma (HCC). Hepatocytes are the original source of malignant tumors, known as HCC. Nearly three-quarters to three-quarters of prostate cancer cases are HCC [1]. Different age groups, geographies, and sexes have significantly different rates of HCC incidence and prevalence. It ranks second in cancer-related fatalities among males and fourth in overall incidence of the disease [2].

Epidemiological studies have shown that the incidence of HCC varies greatly across regions. For example, hepatitis B virus (HBV) infection is more common in Southern Africa and Eastern and Southeastern Asia, which explains why these regions have the highest incidence rates. China, with its massive population, has the highest number of HCC cases, accounting for approximately half of all cases worldwide [3]. Liver fibrosis and cirrhosis are common precursors of HCC, which may be caused by factors such as heavy alcohol use, lipid buildup, chronic viral infections, toxins, and chronic viral infection [4]. Approximately 887,000 people die as a consequence of complications from chronic HBV infection, the leading causes of which are liver cirrhosis and hepatocellular cancer [5]. Viral hepatitis accounts for the vast majority of HCC cases (>90%), with 50% of these cases caused by HBV, especially in highly endemic regions. Hepadnaviruses are a class of tiny, enveloped DNA viruses that preferentially attack hepatocytes.

HBV is a prototypical member of this family. The viral reverse transcriptase, which is present in all hepadnaviruses, can reverse-transcribe the encapsidated pregenomic RNA (pgRNA) and produce relaxed circular DNA (rcDNA), a genome that is partially double-stranded and replicated in the cytoplasm. The enzyme also possesses activities of RNA-dependent DNA polymerase and DNA-dependent DNA polymerase [٦] . A prevalence of 1.6% for hepatitis B surface antigen (HBsAg) was reported in a community-

based investigation in Iraq that included 9610 households. Moreover, 9.7 percent of the population had anti-HBc antibodies and 17% had anti-HBs antibodies. Therefore, HBV is thought to have low to moderate endemicity in Iraq [7]. The resolution or continuation of HBV infection is primarily influenced by the host's immune response. The establishment of chronic HBV infection necessitates a complex interplay between HBV and a deficient immune response[8,9].

Accumulation of several genetic mutations causes HCC, a tumor that is quite diverse. Mutations that enhance metabolic pathways and HCC proliferation are called driver mutations, whereas mutations that do not provide any selective growth benefit are called passenger mutations[10]. One type of genetic variation that might cause disease is known as a single nucleotide polymorphism (SNP) [11]. HCC development has been linked to many single-nucleotide polymorphisms (SNPs) that correspond to various pathways, including inflammation, DNA repair, cell cycle HCs, oxidative stress, iron metabolism, and growth factors [12]. One of the many types of mutations is SNP, which accounts for approximately 90% of the diversity in human DNA. SNPs account for 0.1 percent of the population variation and have been linked to several diseases. DNA modification is the modification of one nucleotide—A, T, C, or G [13].

SNPs are among the most common types of genetic variations in humans. Promoters, exons, introns, and untranslated regions (UTR) such as the 5′ and 3′ UTR are common places for these variants to arise in genomes [14] Half of SNPs in the coding domain are missense, while the other half are silent or synonymous. Non-coding SNPs can alter miRNA stability, promoter function, and gene expression [15]. These nsSNPs mediate almost 50% of the genetic alterations associated with human illnesses[16]. Studying SNPs in the 3'- UTR of genes that have a considerable function in the development and incidence of HCC is still uncommon. The area around the promoter SNP influences gene expression by modifying histone modifications, DNA methylation, promoter activity, and transcription factor binding [17].

Epidermal growth factor receptor (*EGFR*) is a major gene family owing to their crucial regulatory roles in cell biology. They convert ATP to ADP, catalyzing the transphosphorylation of hydroxyl-containing proteins. This initiates intracellular signaling cascades, stimulating the expression of 518 protein kinase genes, accounting for 2% of all human genes [18]. *EGFR* spans 110 kb of DNA separated into 28 exons and is situated in the 7p12-14 area of chromosome 7 short arm q22. The estimated number of *EGFR* receptors per cell in normal cells ranges from 40,000 to 100,000, but cancer cells overexpress over 106 receptors per cell [19]. *EGFR* is a crucial mediator of cell signaling pathways, including mitosis and cancer development, regulating cell proliferation, adhesion resistance, inflammatory response activation, mucus production, and cell motility[20].

The 40 kDa homophilic type I transmembrane glycoprotein, known as epithelial cell adhesion molecule (*EpCAM*) was first identified by screening monoclonal antibodies against antigens produced by colorectal cancer cells. Retinoblastoma, some types of squamous cell carcinoma, and adenocarcinoma are among the primary tumors and metastases that often exhibit upregulated *EpCAM* [21]. *EpCAM* is a stem cell marker in cancer cells that preserves and enhances cell stemness in various malignancies including HCC. Tumors from mesenchymal or neural tissues do not express *EpCAM*, while epithelial cancers such as lymphomas and melanomas show low or no expression[22].

When cells develop into mature hepatocytes, the expression of *EpCAM* decreases, making it an essential marker for hepatic cancer stem cells (CSCs) in HDC. Cancers that express large amounts of *EpCAM* tend to have a worse prognosis [21]. The current research aimed to examine if HBV Iraqi patients who had the -935 C/G SNP (*rs62139665*) in the promoter region of *EpCAM* gene and *EGFR* 3'UTR of SNP (*rs884225*) location were more likely to develop HCC.

METHODOLOGY

Collection of samples:

A case-control study was conducted on the following study groups during the period from the January 2023 to August 2023. This study was approved by the Institute for Genetic Engineering and Biotechnology for Postgraduate Studies/University of Baghdad, and the study protocol was approved by the Ethics Committee of the Iraqi Ministry of Health and Environment. Patients were recruited from the Medical City, Hepatology and Gastroenterology Teaching Hospital, Baghdad, Iraq. A total of 120 samples were collected, 40 newly diagnosed patients with HCC representing **group I**, 40 patients chronic HBV representing **group II** and 40 HC, from the staff members of hospital where the study was performed representing **group III**. Inclusion criteria were as follows:(1) HCC patients with a history of HBV infection in conjunction with clinical imaging and laboratory markers; (2) CHB category, exhibiting symptoms consistent with chronic hepatitis; and (3) HCs.

The exclusion criteria were as follows: liver damage induced by other causes, including kidney, lung, severe heart, drug intake, HCV and HIV infections, and/or systemic diseases, and liver cirrhosis and liver cancer caused by other chronic liver diseases people undergoing dialysis. An oncologist diagnosed HCC and HBV infection in the Iraqi patients.

Study Primers

Primers were constructed using Primer 3plus, V4, and their reference sequences were validated in the NCBI database and via the programs of the University Code of Student Conduct (UCSC). The primers were then double-checked with their reference sequences in the NCBI database All the primer sequences used in the tests as below:

- **EGFR *(SNP Genotyping)* rs884225**: forward primer 5'- TGTCTTCCATTCCATTGTTTTG -3'; reverse Primer: 5'- TGTGTCATCCTCTCTTGCTGA -3'
- ***EpCAM (SNP Genotyping) rs62139665***: forward primer 5'- ACGCCCGGCTAATTTTGTAT-3'; reverse Primer: 5'- AAGTTCGAGACCAGCCTGAC -3'

Preparing the Primers

Primers were lyophilized and dissolved in nuclease-free water, prepared a 100µM stock solution, and diluted with 90µL of nuclease-free water to create a 10µM workable solution, which was stored at -20°C until needed.

DNA extraction

DNA was extracted according to the instructions provided by the ReliaPrepTM Blood gDNA Miniprep System extraction kit (Promega Company, USA). The DNA of patients and healthy individuals was extracted from the blood samples collected in EDTA tubes.

***EGFR, EpCAM* gene amplification**

Human receptor mutagenesis (HRM) was performed on *EGFR* and *EpCAM* genes (rs884225 and rs62139665). The reaction components consisted of a final volume of 20 µl final volume including DNA (3 µl), nuclease-free water (5 µl), forward primer (1 µl), reverse primer (1 µl), and master mix (2xTransStart®Tip Green qPCR Super Mix) (10 µl). The thermal profile of the HRM Technique for analyzing the three polymorphisms consisted of an enzyme activation cycle lasting 30 s at 94 °C, followed by 40 cycles of denaturation lasting 5 s at 94 °C, annealing lasting 15 s at 58 °C, and extension lasting 20 s at 72 °C. Finally, a time-dependent HRM study was carried out at temperatures ranging from 55 °C to 95 ◦C (0.2 degrees, 1 s).

Extraction concentration and agarose gel electrophoresis

The EDTA blood samples of both the patients and the controls were stored at -20C. Genomic DNA was extracted using the ReliaPrepTM Blood gDNA Miniprep Kit. Using a nanodrop assay, we determined the concentration range of the samples and purity of the genomic DNA.

Genotyping by High-Resolution Melting (HRM) analysis

High-Resolution Melting (HRM) analysis with 0.2◦ C scaling from 55 to 95 ◦C and wavelength analysis (470-510 nm) were performed using a Rotor gene Q Real-time CYTO PCR System (QIAGEN) for genetic analysis (polymorphism analysis). Two sets of synthetic SNP sequences were tested using 2xTransStart Tip Green qPCR Super Mix. Allelic differences were determined using qPCR-HRM with three synthetic controls. The HRM Tool, which is part of the integrated software, was used to create normalized melting curves (NMC) and differential curves (DC) (rotor gene 4.4).

Statistical Analysis:

The data were described, analyzed, and displayed using SPSS version 26, a statistical software for the social sciences. The frequencies of alleles and genotypes were examined using the Hardy-Weinberg equilibrium (HWE). Genotype and allele frequencies were compared between the sick and HCs groups using the chi-squared test. To analyze potential correlations among genetic variants of (*EGFR* and *EpCAM*), odds ratios (ORs) with a 95% confidence interval (CI) were calculated to ascertain the strength of the link among the evaluated gene SNPs.

RESULTS

The study population was categorized into three groups: those with HCC (40 patients) and those with chronic HBV (40 patients). Additional (n = 40) HCs. The demographic characteristics of HCC and CHB patients with similar age and sex were recruited as the HCs group. There was no significant variation in the mean age or sex group among males and females ($p < 0.05$) in different groups.

DNA genotyping results

Genotypes result using HRM

Genotyping was performed on SNPs involving *EGFR* and *EpCAM* (rs884225 and rs62139665) using HRM real-time PCR detection. DNA samples were collected from all participants.

Genotype and Allele Frequency of *EGFR Gene rs884225 SNP* in patients with HCC, CHB and HCs

The Hardy-Weinberg equation was used to analyses the distribution of the control group's CC, TC, and TT genotypes of rs884225, and the findings are shown in table (1). The observed distribution of *rs884225* among control subjects did not significantly deviate from the expected distribution under Hardy-Weinberg equilibrium, with a P-value of 0.5.

Table 1: Expected Frequencies of *rs884225TC*

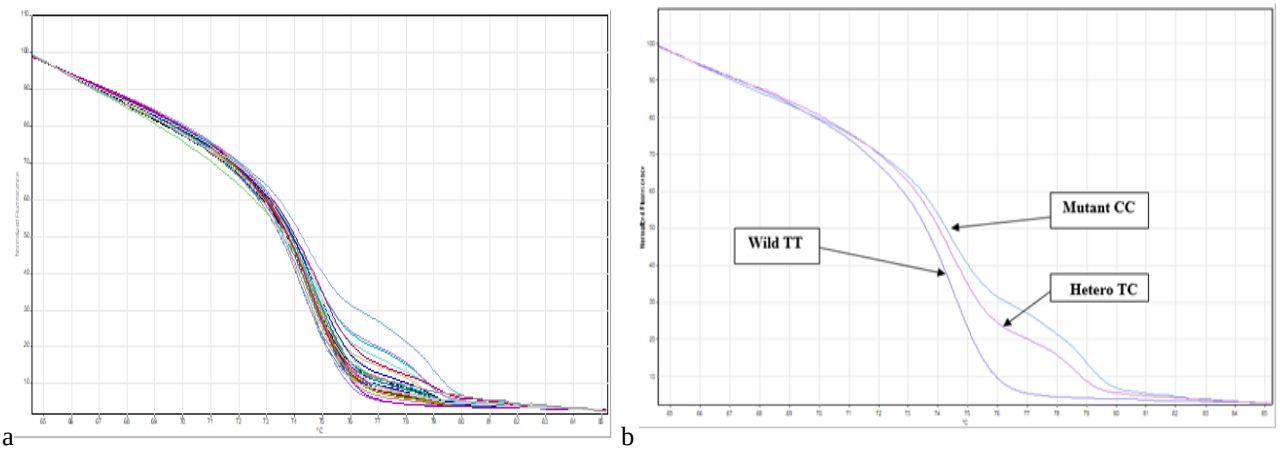
Groups *rs884225*		TT	TC	CC	*p*-value
Control	**Observed no.**	27	11	2	**0.5**
	Expected no.	26.406	12.188	1.406	

The genotypes and allele frequencies of *rs884225* SNP among patients with HCC and HCs are shown in table (2) and the homozygous wild genotype TT was Ref. and the highest percentage were in HCC patients 80% and 67.5% in HCs, while the frequency of the heterozygous genotype (TC) was less frequent in HCC patients (12.5%) than in HCs (27.5%), with an odds ratio (OR) of 0.38 (95% CI: 0.1185–1.241) and a p-value of 0.1. Although the homozygous mutant CC genotype did not show to be a statistically significant risk factor (OR=1.2), according to the allele frequency of *rs884225*, the frequency of the T allele pointed elevated in HCC patients compared to HCs, suggesting that the protective effect of the T allele was 86.3% versus 81.3%, whereas the frequency of the C allele was lower in HCC patients than in HCs and non-significant variation among patients and HCs groups (P> 0.05).

Table 2: Genotype and Allele Frequency among HCC patient groups compared with the healthy group of *EGFR Gene SNP rs884225*

SNP rs884225	**Frequencies (%)**		**X^2**	**Odd ratio (95% CI)**	***p*-value**
	HCC (n= 40)	**HCs (n= 40)**			
Genotype frequency					
TT	32 (80%)	27 (67.5%)	---	1.00 (Reference)	**-**
TC	5 (12.5%)	11 (27.5%)	2.6	0.38 (0.1185 to 1.241)	**0.1**
CC	3 (7.5%)	2 (5%)	0.06	1.2 (0.1968 to 8.138)	**0.8**
Allele frequency					
T	69 (86.3%)	65 (81.3%)	---	1.00 (Reference)	**-**
C	11 (13.7%)	15 (18.7%)	0.734	0.6 (0.2957 to 1.614)	**0.3**

Figure 1 shows the genotype of (rs884225) polymorphism. Figure (a) shows the HRM results for several samples; this picture was taken straight from the device. Figure (b) shows the three SNP genotypes: wild homozygous, heterozygous, and mutant homozygous.



a b

Fig. 1. a: The Human Resource Management output. **b**: This is an image straight from the HRM analysis program showing the three rs884225 genotypes: the wild TT genotype in black, the heterozygous TC genotype in grey, and the mutant CC genotype in green.

The comparison of genotypes and allele frequencies of *rs884225 SNP* among patients with CHB and HCs is shown in table (3). The Homozygous wild-type genotype TT was reported in Ref. and the highest percentages were observed in CHB (72.5% and 67.5%, respectively). The risk analysis showed that the homozygous mutant genotype CC showed non-significant variations with a p value of ≥0.05 and a risk factor (OR=1.3), while the frequency distribution of genotypes among patients and HCs groups varied non-significantly depending on the heterozygous genotype TC (OR= 0.6). This indicates that, compared to individuals with other genotypes, those with a homozygous CC genotype had a nearly one-fold increased risk of developing illness. In addition, sequencing results revealed that the allele frequency of *rs884225* had no significant variations for either alleles T and C among CHB patients and HCs (P< 0.05).

Table 3: Genotype and Allele Frequency among CHB patient groups compared with the healthy group of *EGFR Gene SNP rs884225 T>C*

SNP rs884225	Frequencies (%)		X^2	Odd ratio (95% CI)	P value
	CHB (n= 40)	HCs (n= 40)			
Genotype frequency					
TT	29 (72.5%)	27 (67.5%)	---	1.00 (Reference)	
TC	8 (20%)	11 (27.5%)	0.5	0.6 (0.2368 to 1.936)	0.4
CC	3 (7.5%)	2 (5%)	0.1242	1.3 (0.2164 to 9.010)	0.7
Allele frequency					
T	66 (82.5%)	65 (81.3%)	---	1.00 (Reference)	
C	14 (17.5%)	15 (18.7%)	0.0421	0.9 (0.4110 to 2.05)	0.8

Genotype and Allele Frequency of *EpCAM Gene rs62139665 SNP* in patients with HCC, CHB and HCs.

Table (4) displays the outcomes of applying the Hardy-Weinberg equation to the rs62139665 genotypes, including CC, CA, and AA, as well as their distribution within the control group. Of the 40 HC, 15 had the natural CC homozygous genotype, 24 had the CA heterozygous genotype, and one had the AA mutant genomic variant. There was a statistically significant difference between the predicted and actual distributions of the control participants according to the rs62139665 genotypes (p = 0.02).

Table 4: Expected Frequencies of *rs62139665*

Groups *rs62139665*		CC	CA	AA	*P*-value
Control	**Observed no.**	15	24	1	**0.02**
	Expected no.	18.225	17.550	4.225	

The comparison of genotypes and allele frequencies for the rs62139665C>A SNP among patients with HCC and HCs is shown in table (5). According to genotype frequencies and homozygous wild CC genotype was Ref and the highest percentage was in apparently HCs 37.5% and 15% in HCC, the frequency of the heterozygous CA genotype was not significantly different (p = 0.1, OR = 2.0). The frequency of the homozygous mutant AA genotype was 35% in patients with HCC and 2.5% in HCs. These results suggest that homozygous mutant AA genotypes are associated with an increased risk of developing the disease in the population by AA genotype. This indicates that, compared to individuals with other genotypes, those with the homozygous AA genotype had a 35-fold increased risk of developing illness.

The frequency of the C allele was lower in HCC patients than in apparently HCs (40% versus 67.5%), whereas the frequency of the A allele was higher in HCC patients than in apparently HCs (60% versus 23.5%), and there was significant variation among patients and HCs (P> 0.05) OR=3.1). The study indicates that the presence of the C allele (normal allele) decreases the risk of the disease, while the A allele (mutant allele) may increase the risk in individuals carrying the A allele compared to those without it.

Table 5: Genotype and Allele Frequency among HCC patient groups compared with the healthy group of *EpCAM* Gene SNP *rs62139665C>A*

SNP rs62139665	Frequencies (%)		X^2	Odd ratio (95% CI)	P-value
	HCC (n= 40)	HCs (n= 40)			
Genotype frequency					
CC	6 (15%)	15 (37.5%)	---	1.00 (Reference)	
CA	20 (50%)	24 (60%)	1.688	2.0 (0.6815 to 6.368)	**0.1**
AA	14 (35%)	1 (2.5%)	14.86	35 (3.7304 to 328.383)	**0.0001****
Allele frequency					
C	32 (40%)	54 (67.5%)	---	1.00 (Reference)	
A	48 (60%)	26 (32.5%)	12.16	3.1 (1.6308 to 5.951)	**0.0006****
*** and ** means significant at 0.05 and 0.01 levels respectively.**					

The comparison of genotypes and allele frequencies for *rs62139665* SNP among patients with CHB and HCs is shown in table (6). According to the genotype frequencies, the homozygous wild CC genotype was Ref, and the highest percentage was in HCs (37.5% and 17.5%, respectively CHB patients. The frequency of the heterozygous CA genotype was no significant variations p value = 0.08, OR = 2.5. The homozygous

mutant AA genotype frequency was 12.5% in CHB and 2.5% in HCs and showed significant variations (P≤ 0.05, OR= 10.7). The study indicates that homozygous mutant AA genotypes significantly increase the risk of developing the disease in the population, approximately 12 times higher than other genotypes.

Allele frequency indicated an elevated C wild allele (67.5%) in the HCs group compared with the CHB patient group (52.5%), indicating that the C allele was a protective allele for the HCs group. Elevated A mutant allele was increased in the CHB patients' group (47.5%) compared with the HCs group (32.5%) with an OR of 1.8 (p<0.01), The A allele is a risk allele for CHB patients, with the C allele reducing the disease risk, and the A allele potentially increasing the risk in those carrying the A allele compared to those without the allele.

Table 6: Genotype and Allele Frequency among CHB patient groups compared with the healthy group of *EpCAM Gene SNP rs62139665C*

SNP rs62139665C	**Frequencies (%) CHB (n= 40)**	**HCs (n= 40)**	**X^2**	**Odd ratio (95% CI)**	**p value**
Genotype frequency					
CC	7 (17.5%)	15 (37.5%)	---	1.00 (Reference)	
CA	28 (70%)	24 (60%)	3.0094	2.5 (0.8750 to 7.143)	**0.08**
AA	5 (12.5%)	1 (2.5%)	5.1086	10.7 (1.045 to 109.78)	**0.02***
Allele frequency					
C	42 (52.5%)	54 (67.5%)	---	1.00 (Reference)	
A	38 (47.5%)	26 (32.5%)	3.75	1.8 (0.9894 to 3.568)	**0.05***

*** and ** means significant at 0.05 and 0.01 levels respectively.**

DISCUSSION

Mutations in atypical nucleotide sequence alterations are often, though not invariably, a characteristic that induces disease. A variation in the DNA sequence seen within a population at a frequency of 1 percent or higher is termed a polymorphism [23] Genetic factors such as mutation time and SNP are known to play a considerable role in the onset and progression of HCC. Additionally, the aberrant expression of protein-coding genes has been linked to the development of HCC [24]. One important application of SNPs is the identification of disease-susceptibility polymorphisms. Variations in protein quantity and production rather than quality have resulted from bi-allelic SNPs being more often introduced into genes as a consequence of natural selection. Interactions between ncRNAs and protein-coding genes may cause cell cycle alteration, invasion, differentiation, aberrant cell proliferation, or apoptosis, and ncRNAs are recognized to significantly contribute to the development and occurrence of HCC [25].

Primarily found on epithelial cell surfaces, *EGFR* is a tyrosine kinase transmembrane receptor that belongs to the ERB family of receptors. Researchers have focused on *EGFR* SNPs in HCC because of the strong correlation between these variants and disease risk [26]. According to another study, the *EGFR* (rs884225) polymorphic locus interacts with miR-3196, which has a greater affinity for *EGFR*. This leads to miR-3196 binding to the 3^{a}UTR region of *EGFR*, which in turn inhibits *EGFR* expression and cell proliferation [27]. Through many signaling cascades, including the PI3K-PTEN-Akt and Ras-RAF-MAPK pathways, Ghasimi *et al*.[28] demonstrated that *EGFR* primarily functions in signal transduction of DNA repair, tumor cell survival, and cell proliferation. This is because *EGFR* gene variation is now a focus of intense investigation. To determine whether genetic variations in *EGFR* (rs884225) T/C may be a genetic marker for predicting HCC susceptibility or protection, we used tetra-ARMS-PCR analysis to analyze the genetic correlations of *EGFR* (rs884225) T/C with HCC. According to previous studies [29]. This mutation is associated with DNA amplification and allele copy number variation. The Cancer Genome Atlas (TCGA) contains data regarding *EGFR* somatic mutations. Six *EGFR* mutations have been identified in human tumors. These mutations spread across the entire structure of the *EGFR* gene, including its intracellular and extracellular domains. This suggests that *EGFR* mutations may be involved in the signal transduction process, which includes a combination of ligands and downstream signaling [30].

The findings of this study provide new support for the association between *EGFR* (rs884225) and HCC based on earlier research that found *EGFR* (rs4947986) and rs884225 polymorphisms to be involved in HCC, with functional evidence demonstrating *EGFR* dysfunction [25]. According to Zhang *et al. 's* research on *EGFR* polymorphisms in liver cancer, The *EGFR* rs884225 polymorphism has been associated with a reduced risk of HCC in the Chinese Han population.

Specifically, the TG and GG genotypes of this polymorphism are linked to decreased HCC risk compared to the TT genotype. The polymorphism's impact on tumor size further underscores its potential as a biomarker for HCC risk stratification [31].

Although cirrhosis greatly increases the risk of HCC, it may occur in non-cirrhotic livers and even in the absence of inflammation in some individuals [32]. Genetic and epigenetic abnormalities that cause HCC development have been documented in many studies. The development of HCC is caused by a combination of several genetic changes, and identifying oncogenes that are unique to different types of cancer might open up new avenues for the creation of molecularly targeted treatments [33]. Understanding the role of SNPs in disease susceptibility can inform personalized medical approaches, allowing for tailored therapeutic interventions based on an individual's genetic profile. This is particularly relevant in cancers, such as HCC, where genetic variations can significantly impact treatment outcomes. The *EGFR* pathway is a promising target for HCC therapy, particularly in patients with specific genetic backgrounds. The use of *EGFR* inhibitors and the identification of novel therapeutic targets, such as the internalization-related epitope on *EGFR*, offer potential strategies for treating HCC [34].

Most epithelial malignancies, with the exception of squamous, urothelial, and renal cell carcinomas, have high levels of expression of *EpCAM*, a transmembrane glycoprotein of approximately 40 kDa that is located on chromosome 2p21. Once cells undergo differentiation into mature hepatocytes, *EpCAM*, a crucial marker for hepatic CSCs, disappears [35]. Carcinomas with elevated levels of *EpCAM* are associated with a poor prognosis. *EpCAM* not only serves a vital function in cell-cell connections, but is furthermore engaged in hepatic cell renewal, migration, proliferation, signaling, and differentiation. In order to exert its effects, *EpCAM* activates Wnt signaling and raises c-Myc expression in rapidly dividing tumor cells [36]. This study is the first to establish a link between *EpCAM* rs62139665 and the risk of HCC. It has been shown that transcriptional activity 1.1 kb upstream of the *EpCAM* gene is strongly correlated with *EpCAM* levels, and rs62139665 is situated 935 upstream of the promoter region [37].

The majority of the variation in genetic features across individuals, including susceptibility to illness, prognosis, and response to treatment, is caused by SNPs, which are the most prevalent type of genetic variety found throughout the human genome. Section responsible for promoting Because transcription factors bind to certain nucleotide sequences inside a gene's SNPs, this area controls gene expression, which in turn affects translation and increases a person's risk of developing cancer and other disorders. One example is the *EpCAM* gene, where SNP rs1126497 is strongly linked to a higher risk of breast and cervical cancers [38]. The results of this study suggest that SNPs in the *EpCAM* gene contribute significantly to the development and progression of various cancers.

HCC as other malignancies is attributed to accumulated genetic alterations[39]. Our findings suggest that some genetic variations in the promoter region of *EpCAM*, namely, the (rs62139665) SNP, may increase the risk of HCC in Iraqi individuals. Several polymorphisms in *EpCAM*, including rs112649, rs1421, and rs62139665, have been associated with a decreased risk of HCC in an Egyptian population. [38]. Frequency of *EpCAM* (rs62139665) gene and allele polymorphisms. in the current study in line with research conducted in Egypt [40]. The current findings provide more evidence of a strong link between *EpCAM* (rs62139665) variation and the risk of HCC.

There was substantial variation in the genotype and allelic distribution of the SNP (rs62139665) between HCs and HCC patients. There may be protective benefits of the C allele, since the prevalence of the *EpCAM* AA genotype and A allele was much higher in HCC patients than in HBV patients and HCs. Logistic regression analysis revealed a statistically significant correlation between the AA genotype and the rs62139665 A allele in relation to HCC risk. The current study found that the AA genotype significantly upregulated the expression levels of the *EpCAM* gene and protein, which explains and supports the link with HCC risk. Multiple investigations have linked SNPs in the promoter region to increased gene expression [40].

A multitude of research has demonstrated that the *EPCAM* gene is involved in the development, progression, and recurrence of HCC [41]. The significance of genetic variations, including those within the EpCAM gene, in influencing the susceptibility to (HCC) among patients infected with hepatitis B virus (HBV) necessitates additional exploration. An enhanced comprehension of these correlations may facilitate the advancement of more effective screening methods and tailored therapeutic approaches for populations at heightened risk [42].

CONCLUSION

In conclusion, our investigation demonstrated an association between *EGFR and EpCAM genes* variants and susceptibility to HCC and HCC progression among some Iraqi populations carrying the *EGFR (rs884225 T>C) and EpCAM (rs62139665C>A)* polymorphisms. These results contribute to the growing understanding of the molecular mechanisms underlying HBV-associated HCC and underscore the importance of genetic profiling for identifying high-risk individuals. Future studies with larger cohorts and functional analyses are warranted to validate these findings and explore the potential of EGFR and EpCAM as biomarkers for early diagnosis or as therapeutic targets in HCC management.

REFERENCES

1. Foglia B, Turato C, Cannito S. Hepatocellular carcinoma: Latest research in pathogenesis, detection and treatment. Int J Mol Sci. 2023;24(15):12224.
2. Chidambaranathan-Reghupaty S, Fisher PB, Sarkar D. Hepatocellular carcinoma (HCC): Epidemiology, etiology and molecular classification. Adv Cancer Res. 2021;149:1-61.
3. Zheng S, Chan SW, Liu F, et al. Hepatocellular Carcinoma: Current Drug Therapeutic Status, Advances and Challenges. Cancers (Basel). 2024;16(8):1582.
4. Norman EML, Weil J, Philip J. Hepatocellular carcinoma and its impact on quality of life: A review of the qualitative literature. Eur J Cancer Care (Engl). 2022;31(6):e13672.
5. Mohsen RT, Al-azzawi RH, Ad'hiah AH. Single nucleotide polymorphisms of interleukin-35 subunit genes predict host susceptibility to chronic hepatitis B virus infection among Iraqi patients. Meta Gene. 2020;25:100735.
6. Diogo Dias J, Sarica N, Neuveut C. Early steps of hepatitis B life cycle: from capsid nuclear import to cccDNA formation. Viruses. 2021;13(5):757.
7. Mohsen RT, Al-azzawi RH, Ad'hiah AH. Hepatitis B virus genotypes among chronic hepatitis B patients from Baghdad, Iraq and their impact on liver function. Gene Reports. 2019;17:100548.
8. Mohsen R. Serum level of interleukin-35 in patients with chronic hepatitis B virus infection. 2020.
9. Al-Juboori SKE, Al-Juboori SIK. Hepatocellular Carcinoma Prediction and early Diagnosis of Hepatitis B and C viral infection using miR-122 and miR-223 in a sample of Iraqi patients. Baghdad Sci J. 2023;20(4 (SI)):1520.
10. Ding XX, Zhu QG, Zhang SM, et al. Precision medicine for hepatocellular carcinoma: driver mutations and targeted therapy. Oncotarget. 2017;8(33):55715.
11. Kadhim MM, Shakir AS. CD14 C (-260) T Polymorphism and Blood Levels of the Soluble Endotoxin Receptor CD14, their Association with Risk of Ischemic Stroke in Iraqi Populations. Indian J Forensic Med Toxicol. 2020;14(4):1979-1985.
12. Panneerselvam S, Wilson C, Kumar P, et al. Overview of hepatocellular carcinoma: from molecular aspects to future therapeutic options. Cell Adh Migr. 2023;17(1):1-21.
13. Akhtar M, Jamal T, Jamal H, et al. Identification of most damaging nsSNPs in human CCR6 gene: In silico analyses. Int J Immunogenet. 2019;46(6):459-471.
14. Mohammed MQ, Alwan AH, Almukhtar AA, Al-Saedi MKA. Revealing of TLR-9 gene polymorphisms by qPCR HRM technique and their influence on TLR-9 serum level in acute myeloid leukemia patients: Case-control study. Cytokine. 2024;182:156730.
15. Akhtar M, Khan S, Ali Y, et al. Association study of CCR6 rs3093024 with Rheumatoid Arthritis in a Pakistani cohort. Saudi J Biol Sci. 2020;27(12):3354-3358.
16. Al-Kazaz AA. Genetic polymorphisms of crisp2 gene in association with infertility in Iraqi patients. Iraqi J Agric Sci. 2023;54(3):657-666.
17. Deng N, Zhou H, Fan H, Yuan Y. Single nucleotide polymorphisms and cancer susceptibility. Oncotarget 8, 110635–110649. 2017.
18. Roskoski Jr R. A historical overview of protein kinases and their targeted small molecule inhibitors. Pharmacol Res. 2015;100:1-23.
19. Wee P, Wang Z. Epidermal growth factor receptor cell proliferation signaling pathways. Cancers (Basel). 2017;9(5):52.
20. Hassanein SS, Abdel-Mawgood AL, Ibrahim SA. EGFR-dependent extracellular matrix protein interactions might light a candle in cell behavior of non-small cell lung cancer. Front Oncol. 2021;11:766659.
21. Mohtar MA, Syafruddin SE, Nasir SN, Low TY. Revisiting the roles of pro-metastatic EpCAM in cancer. Biomolecules. 2020;10(2):255.
22. Xiao D, Xiong M, Wang X, et al. Regulation of the Function and Expression of EpCAM. Biomedicines. 2024;12(5):1129.
23. Hussein SA, Al-azzawi RH. Single Nucleotide Polymorphism of Interleukin-28β Subunit Genes Predict Host Susceptibility to Hepatitis C virus (HCV) Infection among Iraqi Patients. Iraqi J Sci. 2024:2385-2396.
24. Zhang WT, Zhang GX, Gao SS. The potential diagnostic accuracy of let-7 family for cancer: A meta-analysis. Technol Cancer Res Treat. 2021;20:15330338211033060.
25. Jiang R, Tang J, Chen Y, et al. The long noncoding RNA lnc-EGFR stimulates T-regulatory cells differentiation thus promoting hepatocellular carcinoma immune evasion. Nat Commun. 2017;8(1):15129.
26. You KS, Yi YW, Kwak SJ, Seong YS. Inhibition of RPTOR overcomes resistance to EGFR

inhibition in triple-negative breast cancer cells. Int J Oncol. 2018;52(3):828-840.

27. Fan Z, Yang J, Zhang D, et al. The risk variant rs884225 within EGFR impairs miR-103a-3p's anti-tumourigenic function in non-small cell lung cancer. Oncogene. 2019;38(13):2291-2304.
28. Ghasimi S, Wibom C, Dahlin AM, et al. Genetic risk variants in the CDKN2A/B, RTEL1 and EGFR genes are associated with somatic biomarkers in glioma. J Neurooncol. 2016;127:483-492.
29. Li B, Ding CM, Li YX, Peng JC, Geng N, Qin WW. Over-regulation of microRNA-133b inhibits cell proliferation of cisplatin-induced non-small cell lung cancer cells through PI3K/Akt and JAK2/STAT3 signaling pathway by targeting EGFR. Oncol Rep. 2018;39(3):1227-1234.
30. Tang Q, Wang Y, Ma L, et al. Peiminine serves as an adriamycin chemosensitizer in gastric cancer by modulating the EGFR/FAK pathway. Oncol Rep. 2018;39(3):1299-1305.
31. Zhang L, Li X, Lu J, et al. The egfr polymorphism increased the risk of hepatocellular carcinoma through the mir-3196-dependent approach in chinese han population. Pharmgenomics Pers Med. 2021:469-476.
32. Desai A, Sandhu S, Lai JP, Sandhu DS. Hepatocellular carcinoma in non-cirrhotic liver: A comprehensive review. World J Hepatol. 2019;11(1):1.
33. Nikolova D, Trajkovska M, Trpcevska EN, Eftimov A, Jovanovik R, Janevska V. Epidermal Growth Factor Receptor immunohistochemical expression in hepatocellular carcinoma without Epidermal Growth Factor Receptor exons 18–21 mutations. Rom J Intern Med. 2022;60(3):153-159.
34. Huang D, Fan Q, Liu Z, et al. An Epitope on EGFR Loading Catastrophic Internalization Serve as a Novel Oncotarget for Hepatocellular Carcinoma Therapy. Cancers (Basel). 2020;12(2):456.
35. Gires O, Pan M, Schinke H, Canis M, Baeuerle PA. Expression and function of epithelial cell adhesion molecule EpCAM: where are we after 40 years? Cancer Metastasis Rev. 2020;39(3):969-987.
36. Wang MH, Sun R, Zhou XM, et al. Epithelial cell adhesion molecule overexpression regulates epithelial-mesenchymal transition, stemness and metastasis of nasopharyngeal carcinoma cells via the PTEN/AKT/mTOR pathway. Cell Death Dis. 2018;9(1):2.
37. Sivagnanam M, Schaible T, Szigeti R, et al. Further evidence for EpCAM as the gene for congenital tufting enteropathy. Am J Med Genet A. 2010;152(1):222.
38. Motawi TMK, Sadik NAH, Sabry D, Shahin NN, Fahim SA. rs2267531, a promoter SNP within glypican-3 gene in the X chromosome, is associated with hepatocellular carcinoma in Egyptians. Sci Rep. 2019;9(1):6868.
39. Fathy WM, Elbassal F, Farouk S, et al. Evaluation of Circulating MicroRNA 483-5p as a Useful Diagnostic Tool of Hepatocellular Carcinoma in Egyptian Patients. Egypt J Med Microbiol. 2018;27(3):45-52.
40. Motawi TMK, Sadik NAH, Sabry D, Fahim SA, Shahin NN. rs62139665 polymorphism in the promoter region of EpCAM is associated with hepatitis C virus-related hepatocellular carcinoma risk in Egyptians. Front Oncol. 2022;11:754104.
41. Krause J, von Felden J, Casar C, et al. Hepatocellular carcinoma: Intratumoral EpCAM-positive cancer stem cell heterogeneity identifies high-risk tumor subtype. BMC Cancer. 2020;20:1-12.
42. An P, Xu J, Yu Y, Winkler CA. Host and viral genetic variation in HBV-related hepatocellular carcinoma. Front Genet. 2018;9:261.