Significance of p53, cyclooxygenase-2, and epithelial cell adhesion molecule expression in hepatocellular carcinoma: an immunohistochemical study

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Background
Hepatocellular carcinoma (HCC) is a significant global health concern with a high mortality rate. To date, the most effective therapy for HCC is resection at an early tumor stage. However, tumor recurrence is common, and identifying key molecules facilitates the understanding of the pathogenesis of HCC and the prediction of prognosis to provide novel targets for anticancer therapy.
Aim
This study evaluated the expression of p53, cyclooxygenase-2 (COX-2), and epithelial cell adhesion molecule (EpCAM) in HCC and investigated their correlation with clinicopathological features and prognosis.
Methods
An Immunohistochemical analysis of p53, COX-2, and EpCAM was conducted on selected 51 HCC cases and adjacent noncancerous hepatic tissue.
Results
In the current study, p53, COX-2, and EpCAM expression were significantly higher in HCC cases than in the adjacent nontumor tissue (P=0.05, P=0.03, and P=0.041, respectively). P53, COX-2, and EpCAM were significantly overexpressed among patients with advanced stage (P=0.039, P=0.000, and P=0.016, respectively), large tumor size (P=0.004 and P=0.001) and poor disease-free survival (P=0.036, P=0.001, and P=0.000, respectively). P53 and EpCAM were significantly correlated with vascular invasion (P=0.045 and P=0.032) and higher grade (P=0.019 and P=0.033). While COX-2 was associated with well-differentiated HCC cases. There was no statistically significant correlation between p53 and COX-2 or, EpCAM, while COX-2 was directly correlated with EpCAM (r=0.001).
Conclusion
p53, COX-2, and EpCAM might have an important role in early carcinogenesis, progression of HCC, and poor prognosis, suggesting that the inhibition of these proteins may hold potential as a multitarget therapeutic approach in HCC patients.

Keywords:
cyclooxygenase-2, epithelial cell adhesion molecule, hepatocellular carcinoma, p53

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Introduction

Hepatocellular carcinoma (HCC), is a significant global health concern, with a high mortality rate of 95%, and is the second leading cause of death from cancer worldwide (Yoshiji *et al.* 2002; Torre *et al.* 2015).

HCC is one of the most common types of malignant tumors in Egypt, and it is the second most prevalent malignancy in men and the fifth in women. As a result, liver cancer is the leading cause of death in Egypt compared with other types of cancer (Strickland *et al.* 2002).

Liver cirrhosis and chronic hepatitis have been recognized as crucial risk factors for the onset of HCC, and the Barcelona Clinic Liver Cancer Staging Classification considers tumor size, vascular invasion, and metastasis to be critical factors in determining clinical staging and prognosis in patients with HCC (Llovet *et al.* 1999).

To date, the most effective therapy for HCC is resection at early tumor stages. However, tumor recurrence is common owing to the limited number of available treatment options. Despite various attempts, no adjuvant treatment is beneficial for HCC, which is attributed

to the high resistance of HCC to chemotherapy and radiation. (Nunes *et al.* 2018).

To enhance the survival prospects of HCC patients, identifying the key molecules and signaling pathways involved in tumor growth, invasion, and metastasis can help elucidate the underlying mechanisms of HCC and provide a novel therapeutic target, which is currently lacking in HCC clinical treatment (Sia *et al.* 2017).

The most commonly implicated gene in the carcinogenesis of HCC is the TP53 suppressor gene, as reported by Graur *et al.* (2016). p53 protein is a potent pro-apoptotic factor that also suppresses cell growth, making it an important protector against cancer development (Lee *et al.* 2013). However, mutations in this gene can lead to the evolution of neoplasms. The prognostic significance of p53 mutations has been reported in certain types of human cancers, such as in a study by Sheen *et al.* (2003).

Cyclooxygenase-2 (COX-2), an enzyme involved in prostaglandin (PG) synthesis that is inducible in response to inflammation, is believed to play a role in carcinogenesis across multiple cancer types, and its expression is linked to cancer cell invasiveness, prognosis, and survival (Esbona *et al.*, 2016). COX-2 overexpression has been observed in HCC, and previous research has suggested that COX-2 promotes tumorigenesis by suppressing p53-mediated apoptosis (Choi *et al.* 2005; Sakr *et al.* 2022).

Epithelial cell adhesion molecule (EpCAM) is a type of protein that is overexpressed in several types of cancer, including breast cancer, glioma, and colorectal cancer (Goossens-Beumer *et al.* 2014). EpCAM plays a role in regulating cancer cell growth and is associated with cancer progression (Gao *et al.* 2017). It has also been found to be abnormally expressed in many cases of epithelial cell cancer, including HCC. Additionally, EpCAM is reported to be downregulated by p53 gene expression. (Litvinov *et al.* 1997; Sankpal *et al.* 2009)

The objective of this research was to examine the expression of p53, COX-2, and EpCAM markers in HCC and to relate them to diverse clinicopathological parameters to clarify their role in hepatocarcinogenesis and prognosis, as they require different interventional treatment modalities.

Patients and methods

Study groups

This retrospective study was conducted using formalin-fixed paraffin-embedded tissue samples from 51 patients diagnosed with HCC and available adjacent nontumorous tissue. Samples were collected from the files and archives of the Pathology Department, Early Cancer Detection Unit (ECDU), Faculty of Medicine, Benha University, between July 2016 and January 2023. This study was approved by the Ethics Committee of the Faculty of Medicine, Benha University (M.S.33.9.2023). The specimens included in the study were either biopsy (20 cases) or surgical resection (hepatectomy) included 31 cases.

The criteria for inclusion were: clinicopathological data obtained from the patients' files, including age, histopathological grade, tumor size, α-fetoprotein level (AFP), cirrhosis presence or absence, and the presence or absence of hepatitis B virus or hepatitis C virus (HCV) infection.

Patients with HCC who had received chemotherapy before the study and those without available paraffin blocks or clinicopathological data were excluded.

Histopathologica1 examination

Formalin-fixed and paraffin-embedded blocks were cut at 5 μm thickness and stained with hematoxylin and eosin. Two observers reviewed the microscopic sections from all the cases and were unaware of their diagnosis.

The HCC cases were graded according to Edmondson and Steiner (Edmondson and Steiner 1954). Patients were staged using the AJCC Cancer Staging Manual 8th edition (American Joint Committee on Cancer 2016) and further divided into early stage (I–II) and advanced stage (III–IV) for statistical analysis. The tumor size was classified according to Tai *et al.* (2019).

Immunohistochemical procedure

For immunohistochemical analysis, the streptavidin-biotin technique was used according to the manufacturer's instructions (Neomarker, LABVISION, USA, CA 94538-7310). The sections were stained with 0.02% diaminobenzidine (DAB) solution as a chromogen. Finally, the sections were counterstained with hematoxylin, dehydrated, and mounted. Negative controls were performed by omitting the primary antibody, and positive controls were added, as shown in Table 1.

Immunohistochemical assessment of p53, COX2, and EpCAM expression in the studied cases

Positive nuclear staining was examined for p53. The number of positive cells was interpreted in adherence to the methodology utilized by Kan and Dong (2015). The percentage of COX-2 and EpCAM -positive cells was scored by Tai *et al.* (2019) and Noh *et al.* (2018). Survival data were plotted as Kaplan–Meier curves and the statistical significance was determined by log-rank test. The follow-up period ranged from 6 to 36 months, with a median follow-up time of 24 months.

Statistical analysis

Results were analyzed using SPSS (SPSS Inc., Chicago, IL, USA) (version 22) statistical package for Microsoft Windows as follows: *P* value greater than 0.05 is no significant (N), *P* less than 0.05 is significant (S), and *P* less than or equal to 0.01 is highly significant (HS).

Results:

Clinicopathological features of the studied cases

This study included 51 cases of HCC. The age of the studied patients ranged from 30 to 80 years, with a mean age of 41 ± 11.7 years. The clinicopathological variables are listed in Table 2.

Immunohistochemical expression of p53, COX-2, and EpCAM in HCC and adjacent nontumorous tissue

Positive nuclear p53 immunostaining was demonstrated in 58.8% of HCC cases and 10% of adjacent noncancerous tissues with a highly significant statistical correlation (*P*=0.005). Higher EpCAM and COX-2 expression were demonstrated in 64.7% and 56.9% of HCC cases, respectively. In nontumorous tissue, 30% and 20% showed high expression of EpCAM and COX-2, respectively, with a statistically significant correlation (*P*=0.041 and *P*=0.03).

Immunohistochemical expression of p53, COX-2, and EpCAM and their relationship with clinicopathological features in the studied cases

p53 expression was positive among patients with higher grade (*P* =0.019), advanced TNM stage

Table 1 Data for using PD-L1, VEGF and CD8 antibodies in studied cases:

Antibody	Type	Source	Dilution	Positive control	Incubation	Antigen retrieval
p53	Monoclonal Mouse/IgG2a	Thermo fisher scientific	1:20	Colon carcinoma	1 h at RT	Citrate buffer (pH 6.0)
COX-2	A mouse monoclonal	Thermo fisher scientific	**1:100**	Pancreatic adenocarcinoma,	**30 min**	**Citrate buffer pH 6.0**
EpCAM	Monoclonal clone VU-1D9,	Thermo fisher scientific	**1:50**	Kidney	**30 min**	**Citrate buffer pH 7.2**

COX-2: Cyclooxygenase-2, EpCAM: Epithelial cell adhesion molecule.

Table 2 Immunohistochemical expression of p53, cyclooxygenase-2, and epithelial cell adhesion molecule and their correlation with the clinicopathological features in the studied cases

Marker Variable		p53		*P* value	COX-2		*P* value	EpCAM		*P* value
		Negative (*N*=21) [*n* (%)]	Positive (*N*=30) [*n* (%)]		Low (*N*=22) [*n* (%)]	High (*N*=29) [*n* (%)]		Negative (*N*=18) [*n* (%)]	Positive (*N*=33) [*n* (%)]	
Age groups (years)	≤60 (29)	13 (44.8)	16 (55.2)	0.543	14 (48.3)	15 (51.7)	0.395	12 (41.4)	17 (58.6)	0.296
	>60 (22)	8 (36.4)	14 (63.6)		8 (36.4)	14 (63.6)		6 (27.3)	16 (72.7)	
Sex	Male (28)	12 (42.9)	16 (57.1)	0.788	11 (39.3)	17 (60.7)	0.540	10 (35.7)	18 (64.3)	0.877
	Female (23)	9 (39.1)	14 (60.9)		11 (47.8)	12 (52.2)		8 (34.8)	15 (65.2)	
Stage	I+II (16)	11 (68.8)	5 (31.3)	**0.002****	9 (56.3)	7 (43.8)	**0.000****	9 (56.3)	7 (43.8)	**0.015***
	III+IV (15)	3 (20)	12 (80)		2 (13.3)	13 (86.7)		1 (6.7)	14 (93.3)	**0.005****
	(20)					**NA**				
Grade	1+2 (32)	18 (56.2)	14 (43.8)	**0.005****	9 (28.1)	23 (71.9)	**0.005****	17 (53.0)	15 (47.0)	
	3+4 (19)	3 (15.8)	16 (84.2)		13 (68.4)	6 (31.6)		1 (5.2)	18 (94.8)	
Size	<5 cm (19)	12 (63.2)	7 (36.8)	**0.014***	13 (68.4)	6 (31.6)	**0.005****	11 (57.9)	8 (42.1)	0.002**
	> 5 cm (32)	9 (28.1)	23 (71.9)		9 (28.1)	23 (71.9)		7 (21.8)	25 (78.2)	
Vascular invasion	Yes (18)	7 (37.9)	11 (62.1)	0.589	6 (31.0)	12 (69.0)	**0.045***	4 (24.1)	14 (75.9)	**0.05***
	No (13)	6 (46.2)	7 (53.8)		8 (61.5)	5 (39.5)		7 (53.8)	6 (46.2)	
	20					**NA**				
HBV	Positive (10)	3 (30.0)	7 (70.0)	0.423	3 (30.0)	7 (70.0)	0.350	2 (20.0)	8 (80.0)	0.259
	Negative (41)	18 (43.9)	23 (56.1)		19 (46.3)	22 (53.7)		16 (39.0)	25 (61.0)	
HCV	Positive (40)	13 (32.5)	27 (67.5)	**0.016***	15 (37.5)	25 (62.5)	0.121	14 (35.0)	26 (65.0)	0.933
	Negative (11)	8 (72.7)	3 (27.3)		7 (63.6)	4 (36.4)		4 (36.4)	7 (63.6)	
Adjacent cirrhosis	Yes (21)	7 (34.3)	14 (65.7)	0.139	8 (40.0)	13 (60.0)	0.503	8 (40.0)	13 (60.0)	0.298
	No (10)	5 (50)	5 (50)		5 (50.0)	5 (50.0)		3 (30)	7 (70)	
	20					**NA**				
AFP	<200 ng (28)	15 (53.6)	13 (46.4)	**0.04*** (	13 (46.4)	15 (53.6)	0.601	13 (46.4)	15 (53.6)	**0.05***
	<200 ng (23)	6 (26.1)	17 (73.9)		9 (39.1)	14 (60.9)		5 (21.7)	18 (78.3)	

AFP, Alpha **fetoprotein**; COX-2, Cyclooxygenase-2; EpCAM, Epithelial cell adhesion molecule; HBV, Hepatitis B virus; HCV, Hepatitis C virus; NA, Not -available.

*significant.

**Highly significant.

(*P*=0.039), large tumor size (*P*=0.004), and HCV positive cases (*P*=0.030), as shown in Fig. 1a, b. There was no statistically significant correlation between p53 expression and the other clinicopathological parameters in the studied cases (*P* >0.05), as illustrated in Table 2.

As shown in Table 2, overexpression of COX-2 in the cytoplasm of tumor cells was closely related to well-differentiated HCC cases rather than poorly differentiated HCC (*P* =0.005) as shown in Fig. 1c and d advanced TNM stage (*P*=0.000), large tumor size (*P*=0.001) and positive vascular invasion (*P*=0.045), with a statistically significant positive correlation.

EpCAM was detected by brown membranous staining of the tumor cells. Tumors with advanced stage (*P*=0.016), higher grade (*P*=0.033) shown in Fig. 2e and f, positive vascular invasion (*P*=0.032), and elevated AFP (*P*=0.001) had a relatively higher expression with significant positive correlations, as shown in Table 2.

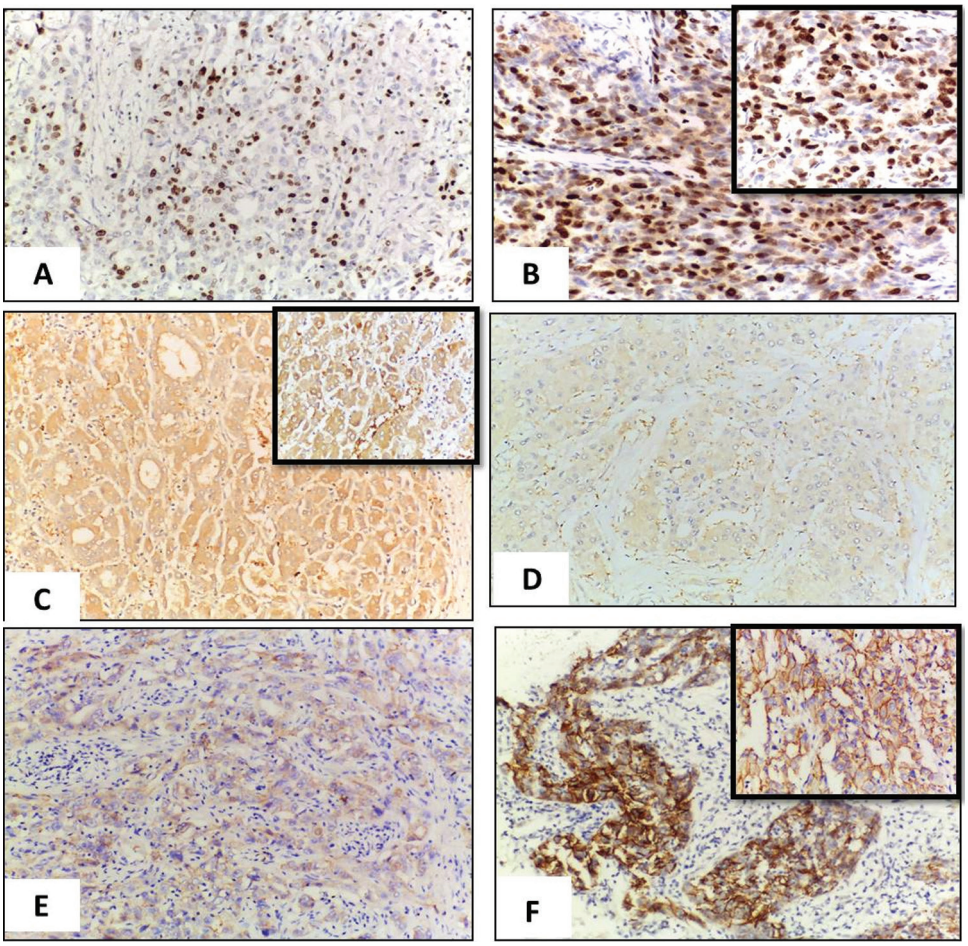
Correlation between p53, COX-2, and EpCAM expression in the studied HCC cases

There was no statistically significant correlation between p53 and COX-2, or EpCAM (*r*=0.902, *r*=0.164). However, there was a significant positive correlation between COX-2 and EpCAM in HCC cases (*r*=0.001), as illustrated in Table 3.

Correlation between p53, COX-2, and EpCAM expression and disease-free survival in the studied HCC cases

The follow-up period ranged from 6 to 36 months, with a median follow-up time of 24 months. According to disease-free survival, 24 (46.7%) patients were disease-free, and 27 (53.3%) patients experienced recurrence/metastasis/or death. A highly significant correlation was found between disease-free survival and p53 (*P*=0.036), COX-2 (*P*=0.001), and EpCAM expression (*P*=0.000) as shown in Fig. 2.

Figure 1



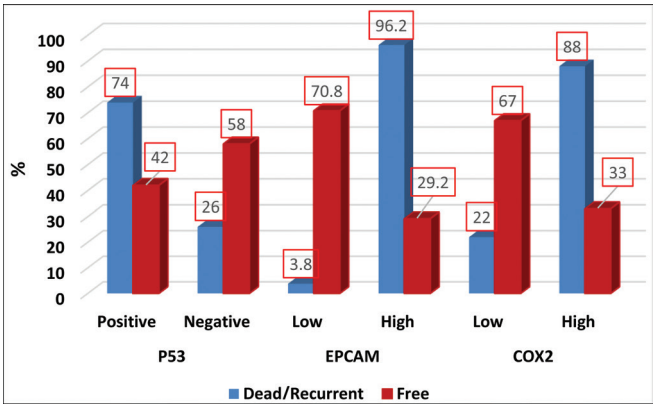
p53, Cyclooxygenase-2, and Epithelial cell adhesion molecule expression in hepatocellular carcinoma. (A) Low-grade hepatocellular carcinoma showing lower nuclear positivity for p53. (B) Diffuse. strong nuclear staining in high-grade hepatocellular carcinoma (IHC, X200, inset x400). (C) Diffuse strong cytoplasmic expression of Cyclooxygenase-2 in moderately differentiated hepatocellular carcinoma|(IHC, X200, inset x400). (D) Focal weak cytoplasmic staining of Cyclooxygenase-2 in low-grade hepatocellular carcinoma. (E) focal membranous staining in low-grade cases (IHC, x200) (F) Diffuse strong membranous expression of Epithelial cell adhesion molecule in high-grade hepatocellular carcinoma cases (IHC, X200, inset x400).

Table 3 Correlation between p53, cyclooxygenase-2, and epithelial cell adhesion molecule expression in the studied hepatocellular carcinoma cases

	P53	EpCAM	COX-2
P53			
Correlation coefficient	–	0.180	0.016
P value		0.164	0.902
EpCAM			
Correlation coefficient	0.180	–	0.380
P value	0.164		**0.001****
COX-2			
Correlation coefficient	0.016	0.380	–
P value	0.902	**0.001****	

COX-2, Cyclooxygenase-2; EpCAM, Epithelial cell adhesion molecule.
**Highly significant.

Figure 2



Correlation between p53, Cyclooxygenase-2, and Epithelial cell adhesion molecule expression and disease-free survival in the studied hepatocellular carcinoma cases.

Kaplan–Meier analysis comparing p53, COX-2, and EpCAM (months) in the studied HCC cases:

Kaplan–Meier analysis showed a significant correlation between p53, COX-2, and EpCAM and disease-free survival ($P<0.05$) and that negative p53 expression and lower COX-2 and EpCAM expression in HCC studied cases is associated with short disease-free survival than cases with positive or higher expression, as shown in Fig. 3.

Discussion

HCC, a liver disorder with multiple risk factors, has been extensively studied in recent years in terms of its prognosis and genetic alterations in patients (Carulli and Anzivino, 2016).

Several investigations have uncovered that certain genes have a considerable impact on the prognosis of individuals diagnosed with HCC. However, the molecular pathways that underlie the formation of HCC are still not well understood. (Llovet *et al.*, 1999). It is essential to discover novel molecular biomarkers in order to comprehend the development and progression of HCC, as well as to predict its prognosis using clinical methods.

In this current study, the expressions of p53 proteins in HCCs were significantly higher than those in non-cancerous tissues (P=0.005). in agreement with our results Luo *et al.* (2001) and Dong *et al.* (2018), first stated that positive nuclear p53 immunostainings were demonstrated in 57.38% of HCC, and 1.69% of adjacent paracancerous tissue. The latter stated that p53 was positively expressed in 60.94% of HCC tissue concluding that p53 has an important role in HCC carcinogenesis.

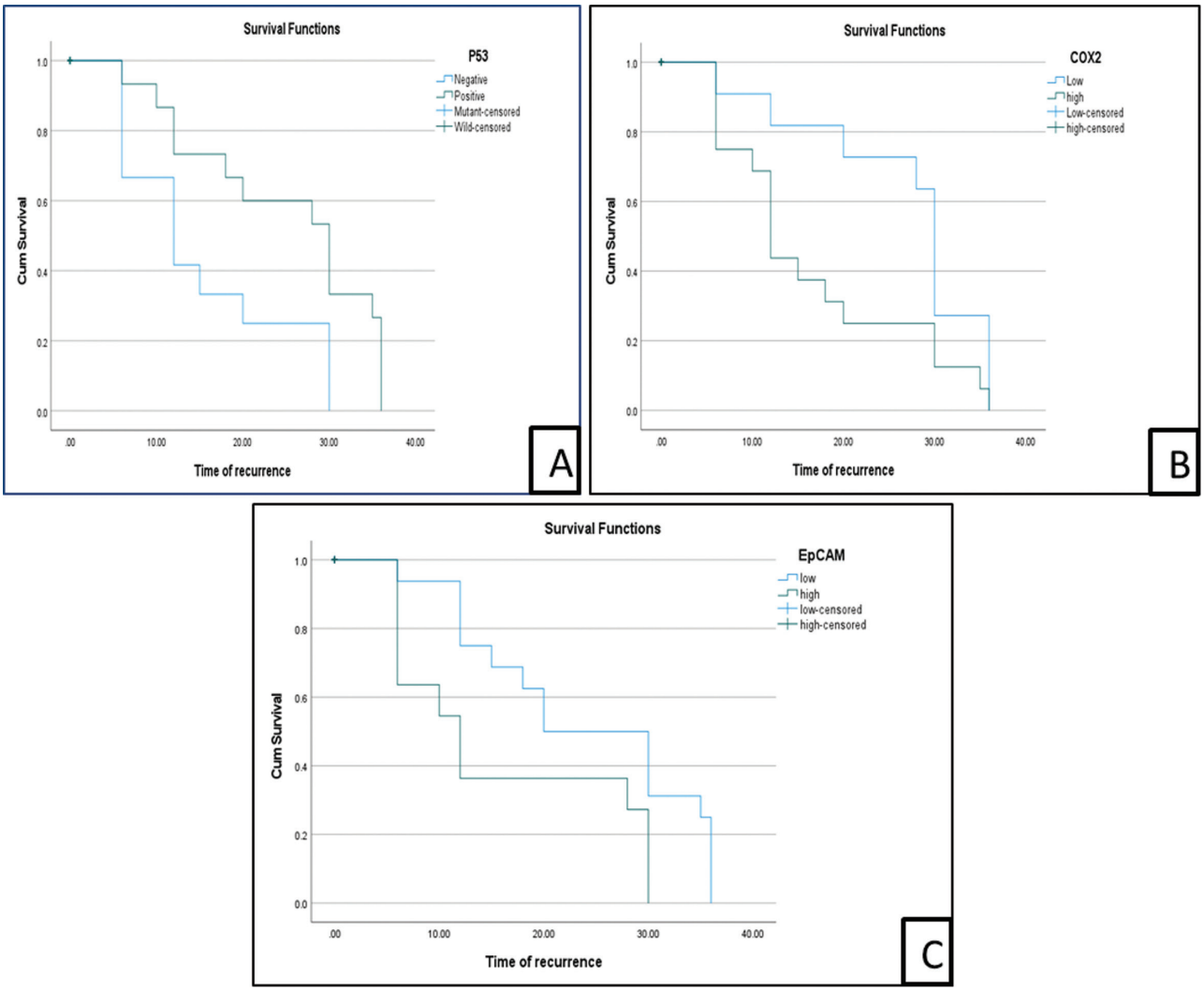
Disagreeing with our results, the study by Sakr *et al.* (2022) that studied the role of p53 expression and the pathological alteration in HCC revealed there was a statistically significant correlation between decreased P53 mRNA expression in HCC groups compared with the healthy control group, which may be due to different genetic alterations of the p53 gene. According to a previous study conducted by Sakr *et al.* wild-type p53 gene expression is downregulated in HCC cases. The study of Liu *et al.* (2016) concluded that the upregulation of the tumor suppressor protein p53 was indeed linked to p53 gene mutations and IHC determination of p53 overexpression can predict p53 gene mutations in HCC.

In this study, a statistically significant positive correlation was found between p53 expression and pathological stage (P=0.002), tumor grade (P=0.005), tumor size (P=0.014), and association with poor disease-free survival (P=0.036). These results were in line with the study conducted by Graur *et al.* (2016), Teramoto *et al.* (1994) and Cheng *et al.* (2004), which pointed out that the only factors that were associated with significant changes in the p53 gene were the degree of tumor differentiation, lymph node invasion, TNM staging, and larger tumor size. The other factors (cirrhosis, HCV, or HBV) were not significantly associated with changes in the p53 gene.

Moreover, in agreement with Sheen *et al.* (2003), who stated that mutant p53 positivity had vascular permeation (P=0.0088), grade II-IV differentiation (P=0.0203), and HCC with p53 mutations recurred more extensively with shorter survival than patients with negative p53 staining.

p53 is a protein that promotes cell death and inhibits cell growth, and it plays a crucial role in regulating the repair and synthesis of DNA, as well as programmed cell death (Lee *et al.* 2013). Once mutated, loss of

Figure 3



Kaplan–Meier estimates of disease-free survival by (A) p53 expression, (B) Cyclooxygenase-2 expression, and (C) Epithelial cell adhesion molecule expression in studied Hepatocellular carcinoma cases.

normal function leads to the evolution of the neoplasm (Sheen *et al.* 2003). This loss of its function is considered a crucial step in carcinogenesis, with the speed of tumor growth and invasion potentially being enhanced. In addition, mutation of the p53 gene has been accentuated in advanced HCC but not in the early stages of HCC (Tanaka *et al.* 1993). P53 might be involved in hepatic carcinogenesis, disease progression, and poor prognosis.

Regarding COX-2 in this present work, it was overexpressed in HCC cases in comparison to control cases with significant differences (P=0.0). In agreement with Sweed *et al.* (2022) which concluded that COX-2 expression was observed at significantly higher levels in HCC tissues compared with normal liver and adjacent nontumor liver tissues.

These results were in association with the study of Sakr *et al.* (2022) that studied the role of COX-2 expression and the pathological alteration in HCC and revealed that a significant correlation of COX-2 expression in HCC groups compared with the healthy control group. Moreover, COX-2 was overexpressed in lower-grade HCC cases with a statistically significant direct correlation (P=0.005). These results were also in line with a study of Cheng *et al.* (2004) showed that well-differentiated HCC expressed more COX-2 than poorly differentiated cancers (P<0.001).

Through the potential activation of the transforming growth factor-a/epidermal growth factor receptor pathway, COX-2 plays a crucial role in the development of HCC (Guerrant *et al.* 2016). TGF-a and EGFR expressions are seen to be elevated in well-differentiated human HCC and lowered in less-differentiated cancers, according to Nikolova *et al.* (2018).

The second hint to explain the decreased expression of COX-2 upon the dedifferentiation of HCC was the form of COX-2 expression in both ductular reactions

and the canal of Hering. Hepatocytes at the level of the canal of Hering were positive for COX-2, but biliary epithelial cells at the same level were not; these results suggested that COX-2 played a role in hepatocyte differentiation, and, therefore, a decreased COX-2 expression may imply a phenotype different from hepatocyte differentiation (Dong *et al.* 2018).

Double hepatic blood supply influences the hemodynamic state during hepatocarcinogenesis because early HCC, which usually corresponds to well-differentiated cancer with few arterial tumor vessels and pre-existing portal tracts, may be greatly affected by these COX-2 inducers via the portal blood flow, leading to strong expression of COX-2. Conversely, advanced HCC, which is characterized by moderately or poorly differentiated HCC, lacks the pre-existing portal tracts that lose highly expressed COX-2 (Schmitz *et al.* 2009).

So, the upregulation of COX-2 in HCC cases rather than adjacent noncancerous tissue and its overexpression in well-differentiated HCC but decreased in poorly differentiated cases, suggesting its crucial role in the early steps of hepatocarcinogenesis.

In this study, a significant statistical positive correlation was found between COX-2 and advanced pathological stage (*P*=0.000), larger tumor size(*P*=0.001), vascular invasion(*P*=0.045) and short disease-free survival (*P*=0.003). These results were in line with the study done by Tai*et al.* (2019) that stated that High COX-2 expression was associated with more tumor aggressiveness.

COX-2, an enzyme that catalyzes the conversion of arachidonic acid to prostaglandins (PGs), is crucial for inflammation and cancer development. Excessive production of the COX-2 metabolite, PGE2, can stimulate the growth, survival, and invasion of epithelial cancer cells (Xie *et al.* 2009). COX-2 can promote the proliferation and angiogenesis of HCC cells via p53, p27, and vascular endothelial growth factor and inhibits apoptosis by inducing the anti-apoptotic factor Bcl-2 and activating the antiapoptotic signaling pathway through the protein kinase B pathway. These effects enhance the invasion and metastasis of HCC cells (Tang *et al*. 2005; Ebsona *et al*. 2016).

Preclinical studies have shown that inhibiting COX-2 can suppress the growth of human liver cancer cells in vitro and reduce the occurrence of liver cancer in animal models (Li *et al.*, 2016). These findings suggest that COX-2 plays an important role in liver cancer development, and targeting COX-2 may be a promising therapeutic strategy for treating liver cancer. These results were against the study of Kondo *et al.* (1999) that showed that there was no association between COX-2 expression in HCC and prognosis. This may be due to the low number of cases, different antibodies, and scoring systems used.

In the current study, the expression of EpCAM proteins in HCCs was significantly higher than that in noncancerous tissues (*P*=0.001). In agreement with our results Ko *et al.* (2018), demonstrated that the expression of EPCAM was significantly higher in HCC tissue than in adjacent normal liver tissue (*P*= 0.001), so this marker may be linked to HCC tumor onset and/or progression.

In this study, a significant statistical correlation was found between EpCAM expression and pathological stage (*P*=0.016), tumor grade (*P*=0.033), vascular invasion (*P* =0.032), AFP (*P*=0.001), and short disease-free survival (*P*=0.000).

These results were in line with the study done by Noh *et al.* (2018) that demonstrated that the overexpression of EpCAM was associated with the tumor stage (*P*=0.05), AFP (*P*=<0.001), Microvessel invasion (*P*=0.003), Edmonson grade (*P*=0.002). Also, in line with a study by Ko *et al.* (2018) and Zhou and Zhu (2018) that showed that the overexpression of EpCAM was associated with the clinicopathological features of HCC, including poorer tumor differentiation and high AFP levels and overexpression of EpCAM was confirmed as the unfavorable predictor for the shorter survival for HCC patients.

Yamashita *et al.* (2013) found that EpCAM-positive cancer cell subpopulations in HCC have the potential for self-renewal, de-differentiation, tumor initiation, invasiveness, and the ability to form distant metastases. Additionally, EpCAM is increasingly being demonstrated to play a crucial role in the epithelial-mesenchymal transition (EMT), which promotes the invasion and progression of tumors (Deng *et al.* 2021).

EpCAM has been shown to regulate the effects of epidermal growth factor on the migration of human ovarian cancer cells (Fan *et al.* 2015) and has been linked to the promotion of prostate cancer metastasis through the phosphatidylinositol 4,5 bisphosphate 3 kinase/Akt/mechanistic target of the rapamycin signaling pathway (Ni *et al.* 2013).

Because of its high antigenicity and expression, catumaxomab, which is an antibody targeting EpCAM, has been used in the treatment of malignant ascites in epithelial cancers (Linke *et al.* 2010). EpCAM has

been proven to be an important pathological marker for patients with cancer and has revealed potential therapeutic opportunities.

In this study, there was no statistically significant correlation between p53 expression and COX-2 and/or EpCAM (P=0.9 and P=0.164, respectively). This supports Sankpal *et al.* (2009) finding that no variation in EpCAM expression was seen after transfection of breast cancer cells with mutant p53.

However, Choi *et al.* (2005) proposed that COX-2 can promote tumor growth by suppressing p53 activity, which triggers cell apoptosis, and the anti-tumor effects of COX-2 inhibitors may result from the enhancement of p53-induced cell death.

This conflict may be attributed to different genetic alterations of the p53 gene, as it was found to be mutated in our study, which was identified by its overexpression in HCC cases, while in the aforementioned study, it was associated with wild-type p53, not mutant one. This is consistent with a meta-analysis conducted by Liu *et al.* (2016), which found that upregulation of the p53 tumor suppressor protein was linked to p53 gene mutations, and IHC determination of p53 overexpression could predict p53 gene mutations in HCC.

This study showed a significant correlation between COX-2 and EpCAM expression (P=0.001), this was in line with Gao *et al.* (2017) demonstrated a positive correlation between EpCAM and COX-2 expression in breast cancer, (P=0.009); These findings imply that EpCAM expression may regulate COX-2 expression in human cancers, and that different subtypes of COX-2-positive carcinomas may respond to therapies that target EpCAM.

Conclusion

Our current research has revealed that the overexpression of p53, COX-2, and EpCAM in cases of HCC differs from that in adjacent noncancerous tissue- suggesting their role in hepatocarcinogenesis. In addition, our findings propose their important roles in the development and progression of HCC and poor prognosis. The potential role of EpCAM in COX-2 regulation recommend that inhibition of these proteins may be a promising multitarget therapeutic approach for patients with HCC.

Recommendation

A comprehensive evaluation of the relationship between p53, COX-2, and EpCAM in HCC is essential to gain deeper insight into their function and potential therapeutic benefits for patients.

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NA

Authors' contributions

Dr O.Y.B. and Dr E.A.S. developed the concept and design and wrote the main manuscript. Dr O.Y.B. and Dr E.A.S. examined and revised cases and methods, interpreted slides, and prepared figures. Dr H.A.E. was involved in the acquisition of data and statistical analysis. The author(s) read, revised, and approved the final manuscript.

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