Original paper

Expression of cyclo-oxygenase-2 and YAP/TAZ in hepatocellular carcinoma in untreated and treated hepatitis C virus patients

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The pathogenesis of hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC) differs according to whether prior treatment with interferon (IFN) vs. direct-acting antiviral agents (DAAs) was administered. Cyclooxygenase-2 (COX-2), yes-associated protein 1 (YAP), and transcriptional co-activator with PDZ-binding motif (TAZ) play a crucial role in hepatocarcinogenesis. However, their roles in untreated or treated HCV-related HCC development have not been clarified.

Therefore, we performed an immunohistochemical study and stained tissue from 83 HCV-related HCC cases using antibodies against COX-2, YAP, and TAZ and correlated their expression with the clinicopathological characteristics and survival data.

The cases were subdivided into 3 groups based on prior HCV treatment. In the 3 groups, COX-2 was significantly higher in HCC tissue compared with adjacent non-tumour liver tissue. However, the expression of YAP/TAZ was not significantly different between HCC and adjacent non-tumour tissue. We further grouped HCC cases into YAP+/TAZ+ and YAP–/TAZ– cases.

In the YAP+/TAZ+ cases, COX-2 was significantly associated with tumour size, tumour multifocality, and late pathologic stage. No significant difference was observed in COX-2 and TAZ expression as a result of IFN or DAA treatment; however, YAP was significantly higher in IFN-treated HCC. Cyclo-oxygenase-2 overexpression may play a role in late HCC development, while YAP/TAZ could play an early role in HCC progression. Sustained expression of combined YAP/TAZ could mediate the poor prognostic role of COX-2.

Key words: COX-2, HCV, hepatocellular carcinoma, TAZ, YAP.

Introduction

The incidence of hepatocellular carcinoma (HCC) is still increasing worldwide, and this cancer represents the fourth most common cause of cancer-related death worldwide [1]. Hepatitis C virus (HCV) infection has been reported to be the most common cause of HCC in Egypt [2]. Most patients with HCC are diagnosed at an advanced stage, and even early-stage HCC is associated with a high recurrence rate after surgical intervention [3]. Therefore, understanding the pathogenesis of HCV-related HCC

is key to a better selection of practical, therapeutic strategies.

Interferon-alpha (IFN-α) and its analogues were, historically, the only treatment modality for HCV, but they have a low cure rate [4]. The newly emerging direct-acting antiviral agents (DAAs) have had an incredible impact in eliminating HCV infection and liver-related mortality in 95% of patients [5]. However, the risk of HCC persists due to background liver cirrhosis, associated cofactors, or potential molecular programming that drives HCC development after viral clearance by DAAs [6].

Cyclo-oxygenase-2 (COX-2) is an enzyme responsible for the generation of prostanoids that contribute to the modulation of multiple procarcinogenic effects [7].

The Hippo pathway is a signal transduction pathway regulated by 2 downstream core proteins: yes-associated protein 1 (YAP) and its transcriptional co-activator with PDZ-binding motif (TAZ). Activated (non-phosphorylated) YAP/TAZ localizes to the nucleus and binds to the TEA domain transcription factors (TEAD) to regulate target gene expression [8]. The role of the Hippo pathway in HCC development is still controversial, as some evidence indicates that YAP/TAZ may have divergent functions [9].

Recent studies have reported a regulatory loop between YAP and COX-2 in human cancers, which mediates tumour invasion, metastasis, and drug resistance [10, 11]. Therefore, revealing the impact of HCV-related treatment in the expression of COX-2 and YAP/TAZ, and understanding the regulatory role of YAP/TAZ expression as well as how it affects COX-2 and its prognostic effects in HCC could be helpful in the selection of patients who would benefit from COX-2 and/or YAP inhibitors.

Material and methods

Samples were collected from the Pathology Department archive. Clinical parameters, laboratory data, and patients' overall survival (OS) data were collected from the medical records. Overall survival (in months) was calculated from the date of diagnosis to the time of death or the date of the last follow-up visit.

Formalin-fixed, paraffin-embedded specimens included 83 HCV-related HCC cases with available adjacent non-tumour liver tissues as well as 10 normal liver tissues (from a liver transplant donor) obtained from Egyptian subjects. All treated HCV patients (60 cases) enrolled in the current study received IFN or DAA therapy prior to HCC development, achieved sustained virological response (SVR), as determined by polymerase chain reaction, and were followed up for 12 months after treatment completion.

The time to HCC development after achieving SVR ranged 2–48 months.

The pathological data included tumour size and focality, tumour pathological grade, lymphovascular invasion, pathological stage according to the 5th edition of the World Health Organization classification of liver tumours and the 8th edition of the American Joint Committee on Cancer staging system [12, 13]. For statistical purposes, tumour size was divided into < 5 cm and ≥ 5 cm groups. In addition, HCC cases were divided into early-stage (T 1–2) and late-stage (T 3–4) pathology groups [14].

Normal liver tissue samples were re-evaluated using routine haematoxylin and eosin staining to confirm the lack of any significant fibrosis, necroinflammatory activity, and steatosis. Furthermore, 4–5-μm-thick tissue sections were cut from each sample, placed on positively charged slides, and used for immunohistochemistry.

Tissue microarray construction

Tissue microarray (TMA) blocks were manually prepared from tumour cases using a 2 mm tissue arrayer needle set (Breecher Instrument, USA). At least 2 representative tissue cores from tumour tissues and one core from non-tumour tissue were included.

Immunohistochemistry

A mouse monoclonal COX-2 antibody (Ref. 187379) was obtained from Invitrogen (California). Rabbit polyclonal YAP (sc-15407) and TAZ (sc-48805) antibodies were obtained from Santa Cruz – Biotechnology Inc. (USA). Sections were placed in high-pH Tris-EDTA solution (Dako, Ref K8000, Glostrup, Denmark) for 20 minutes of heat-mediated antigen retrieval. Sections were incubated with primary antibodies diluted in DAKO antibody diluent at the following concentrations: COX-2 (1 : 100), YAP (1 : 75), and TAZ (1 : 50); slides were incubated overnight at 4°C. Pancreatic adenocarcinoma, kidney, and gall bladder sections served as positive controls for COX-2, YAP, and TAZ, respectively. A negative control was also included.

Assessment of antibodies

Two methods of assessment were applied according to previously published protocols [15, 16]. The first was based on positive/negative expression, where positive expression was considered if any number of hepatocytes showed positive cytoplasmic staining for COX-2 and nuclear staining for TAZ. For YAP, nuclear staining in more than 10% of cells was considered positive. The second method was the Histoscore (H-score) system, which was applied to all cases and was calculated by multiplying the staining intensity (0–3) by the percentage of stained cells, with a final score ranging 0–300.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) program for Windows, version 20 (SPSS Inc., Chicago, Illinois, USA). The Mann-Whitney *U* test was used to compare 2 quantitative variables, while the Kruskal-Wallis test was used to compare more than 2 variables; one-way ANOVA was used to compare one qualitative variable and one quantitative variable, and the Pearson χ^2 test was used to compare qualitative data. A two-tailed *p*-value was considered statistically significant when ≤ 0.05. Kaplan-Meier plots and log-rank tests were used to evaluate OS data.

Results

Ninety-three cases were included in this study: 10 normal cases and 83 HCV-related HCC cases plus corresponding adjacent non-tumour tissues. The median age of all patients with HCC was 58 years, and cases were predominantly male (80.5%). The median serum α-fetoprotein (AFP) level was 47 ng/ml. Nearly all HCC cases (74.5%) also had liver cirrhosis. Most cases of cirrhosis were of moderate pathological grade (82.8%) and early pathological stage (87.4%). The patients with HCC were allocated to 3 groups depending on prior HCV treatment:

- Group 1 included 23 patients with HCC and no previous HCV treatment,
- Group 2 included 16 patients with HCC, who were negative for HCV after IFN treatment,
- Group 3 included 44 patients with HCC, who were negative for HCV after DAA treatment.

No significant difference was observed in the clinicopathological parameters among the 3 HCV-related HCC groups, as shown in Table I. Similarly, no significant difference was identified in the pathological features between HCC and adjacent non-tumour liver tissues), as shown in Supplement (Table I).

Expression of COX-2, YAP, and TAZ in normal liver, adjacent non-tumour, and hepatocellular carcinoma tissues

The expression of the 3 markers in each group is illustrated in Figure 1.

In normal liver tissue, COX-2 expression was cytoplasmic and was positive in 30% of cases. The mean H-score ±SD was 17 ±33.35. In adjacent non-tumour tissue, COX-2 expression was positive in 92.9%, 66.7%, and 72.7% in groups 1, 2, and 3, respectively.

In tumour tissue, COX-2 showed almost equal expression of 95.7%, 100%, and 95.2% in groups 1, 2, and 3, respectively.

Expression of both YAP and TAZ was nuclear and negative in all normal liver tissues, with a mean H-score of 0 ±0. YAP expression in non-tumour tissue showed the highest percentage in the IFN-treated HCV group, while in tumour tissue, YAP protein was expressed in 75% of cases in both treated HCV groups (2 and 3) and in 43.5% of cases in the untreated HCV group. However, TAZ showed the highest expression in both non-tumour and tumour tissues from the untreated HCV group and was expressed in 31.8% and 65.2% of cases, respectively (Fig. 2).

Comparison of COX-2, YAP, and TAZ expression among normal, adjacent non-tumour, and tumour tissues in the 3 HCV-related hepatocellular carcinoma groups

Detailed comparative expression is illustrated in Table II.

In all 3 groups, COX-2 expression was not significantly different between adjacent non-tumour liver tissue and normal liver. Cyclo-oxygenase-2 expression was observed at significantly higher levels in HCC tissues from the 3 HCV groups compared with normal liver and adjacent non-tumour liver tissues irrespective of prior HCV treatment.

YAP was significantly overexpressed in adjacent non-tumour and HCC tissues in the 3 HCC groups compared with normal liver tissue. Furthermore, YAP was overexpressed in HCC tissue in the DAA-treated group compared with adjacent non-tumour tissue ($p = 0.02$). However, no significant difference was observed between HCC and adjacent non-tumour tissues in the IFN-treated and untreated HCV groups.

In the untreated HCC group, significant TAZ overexpression was seen in adjacent non-tumour tissue compared with normal liver. However, no significant difference was observed between adjacent non-tumour tissue and normal liver in both treatment groups. In addition, TAZ was significantly overexpressed in HCC tissues of the 3 HCC groups compared with normal liver tissue. Furthermore, no significant difference was found between TAZ expression in tumour tissues and adjacent non-tumour tissues in the 3 groups.

Comparative expression of COX-2, YAP, and TAZ among the 3 HCC groups is shown in Table III. In HCC cases, YAP was significantly overexpressed in the IFN-treated group compared with the DAA-treated and untreated HCV groups ($p = 0.03$), as shown in Table II. In adjacent non-tumour tissues, no significant difference was observed in COX-2, YAP, or TAZ expression. Therefore, the impact of HCV treatment did not affect their expression in non-tumour liver tissue.

Association between the studied markers and the clinicopathological parameters of hepatocellular carcinoma cases

In all HCV-related HCC groups, almost no significant association was observed between COX-2, YAP, or TAZ expression and clinicopathological prognostic parameters, as shown in Supplement (Tables II–IV).

Table I. Comparison between the clinicopathological data in the 3 hepatocellular carcinoma groups

Parameters	No treatment $N = 23$	IFN $N = 16$	DAAs $N = 44$	Test	*p*-value
Median age					
≤ 60	14	9	33	2.26	0.33
> 60	9	7	11		
Sex					
Male	19	11	37	1.847	0.39
Female	4	5	7		
Median AFP [ng/ml]					
≤ 200	11	8	29	2.77	0.25
> 200	9	6	10		
Tumour focality					
Solitary	17	14	29	2.77	0.25
Multiple	6	2	15		
Median tumour size					
≤ 5 cm	15	7	28	2.26	0.32
> 5 cm	8	9	16		
Pathological grade					
I	1	0	5	5.32	0.26
II	18	14	37		
III	4	2	2		
Pathological stage					
Early stage	23	14	35	5.51	0.06
Late stage	0	2	9		
Lymph vascular invasion					
Negative	11	10	27	1.31	0.52
Positive	12	6	17		
Recurrence					
Free	17	12	30	0.389	0.82
Yes	6	4	14		

AFP – α-fetoprotein, DAAs – direct acting anti-viral agents, IFN – interferon, N – number of patients

The only statistically significant association was identified in group 3. A relationship was found between negative TAZ expression and low AFP level ($p = 0.041$), as shown in Supplement (Table IV).

In addition, no statistically significant association was found between any of the studied markers and the OS of patients. However, old age and non-cirrhotic liver were associated with short OS in HCC cases ($p = 0.027$ and $p = 0.057$), as shown in Supplement (Table V).

The association between COX-2, YAP, and TAZ expression in the HCV-related hepatocellular carcinoma groups

Cyclo-oxygenase-2 expression was not significantly associated with either YAP or TAZ expression in HCV-related HCC. Similarly, no significant association was found between YAP and TAZ expression, as shown in Supplement (Table VI).

The association between COX-2 expression and combined YAP/TAZ expression in HCV-related hepatocellular carcinoma cases

Cyclo-oxygenase-2 was expressed in almost all HCC cases, and its expression was not significantly associated with any clinicopathological parameters. Therefore, we investigated the impact of combined YAP/TAZ expression on the prognostic role of COX-2. All HCV-related HCC cases were allocated into 2 subgroups depending on combined YAP/TAZ expression: YAP^+/TAZ^+ (16 cases) and YAP^-/TAZ^- (20 cases). In YAP^+/TAZ^+ HCC cases, high COX-2

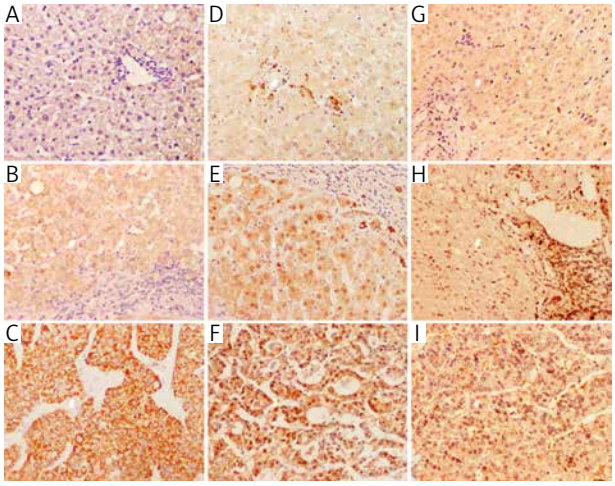

Fig. 1. Comparative immunohistochemical expression of cyclo-oxygenase-2 (COX-2), yes-associated protein 1 (YAP), and transcriptional co-activator with PDZ-binding motif (TAZ) in normal liver, adjacent non-tumour tissue, and hepatocellular carcinoma (HCC) tissues. A) Negative expression of COX-2 in normal liver. B) Mild expression of COX-2 in adjacent non-tumour liver tissue. C) Strong expression of COX-2 in HCC. D) Negative expression of YAP in normal liver. E) Focal nuclear expression of YAP in adjacent non-tumour liver. F) Strong diffuse nuclear expression of YAP in HCC. G) Negative expression of TAZ in normal liver. H) Mild nuclear expression of TAZ in adjacent non-tumour liver. I) Strong and diffuse expression of TAZ in HCC (IHC 200×)

expression was significantly associated with tumour multifocality, large tumour size, and advanced tumour stage ($p = 0.05$, $p = 0.03$, and $p = 0.03$, respectively) (Table IV). However, no significant association was observed between COX-2 expression and clinicopathological data in YAP^-/TAZ^- HCC cases.

Discussion

Cyclo-oxygenase-2 and YAP/TAZ have been reported to be associated with HCC and are frequently upregulated during tumourigenesis [16, 17]. However, the role of HCV treatment in activating the COX-2 and YAP/TAZ pathways in HCV-related HCC has not been reported. It is crucial to understand the effect of HCV treatment on the pathogenesis of HCV-related HCC. This study aimed to highlight the expression of COX-2 and YAP/TAZ in HCV-related HCC and adjacent non-tumour liver tissue in treated and untreated cases, and to illustrate the influence of YAP/TAZ expression on the prognostic effect of COX-2 in HCC.

In the current study, no significant difference was identified between the impact of IFN regimens and DAAs therapy on the clinicopathological parameters of HCC. Patient age and associated cofactors that modulated the prognosis were similar in all groups. However, previous studies have linked late HCC pathological stage following DAA therapy with an infiltrative growth pattern and multiple tumour nodules occurring on top of cirrhotic liver [18].

In this study, normal liver tissue exhibited low COX-2 expression, but no significant difference was observed between COX-2 expression in normal liver tissue and adjacent non-tumour liver tissue in the 3 HCC groups. This was in agreement with the findings of Zidar *et al.*, who reported a complex expression of COX-2 and COX-1 isoforms in normal liver tissue; however, these proteins differed in their distribution. COX-2 was expressed predominantly in hepatocytes, while COX-1 was expressed in blood vessels, smooth muscle cells, Kupffer cells, and resident inflammatory cells [19]. Conversely, other studies reported that chronic HCV infection induced COX-2 overexpression through several pathways to maintain the processes of viral replication, liver fibrogenesis, cirrhosis, and HCC [20]. This discrepancy could be explained by a considerable variation in COX-2 protein expression among cases. Moreover, COX-2 was upregulated at the post-transcriptional level, which limits the accuracy of the real-time polymerase chain reaction technique in the assessment of the COX-2 expression level [19].

YAP/TAZ was not expressed in normal liver tissue in this study. Negative expression indicates that these markers are inducible and are upregulated only under pathologic conditions [21]. This is supported by a significantly higher expression of YAP in adjacent non-tumour tissues in the 3 HCC groups. TAZ showed similar results in the untreated HCV HCC group, while its overexpression in HCV-treated HCC cases was not significant. These data are in agreement with the findings of Abdallah *et al.*, who reported significant overexpression of YAP and TAZ in chronic viral hepatitis compared with normal liver, and that their expression was significantly associated with the stage of fibrosis, inflammatory activity, and bile duct proliferation [22]. It was shown that activation of YAP/TAZ in chronic HCV infection mediates stem cell activation and inhibits hepatocyte apoptosis, which are the key elements in the development of hepatic fibrosis [23].

In the present study, COX-2 was significantly overexpressed in the 3 HCC groups compared with adjacent non-tumour tissue, similarly to previously published studies [24]. Therefore, COX-2 could be a late event in the process of carcinogenesis. Cyclo-oxygenase-2 is a potential combinational target for the treatment of HCC and may play a small role in primary prevention. Conversely, no significant difference was observed in YAP expression between HCC tissue in groups 1 and 2 and adjacent non-tumour liver tissue. Similarly, no difference was found in TAZ expression between the 3 HCC groups and adjacent

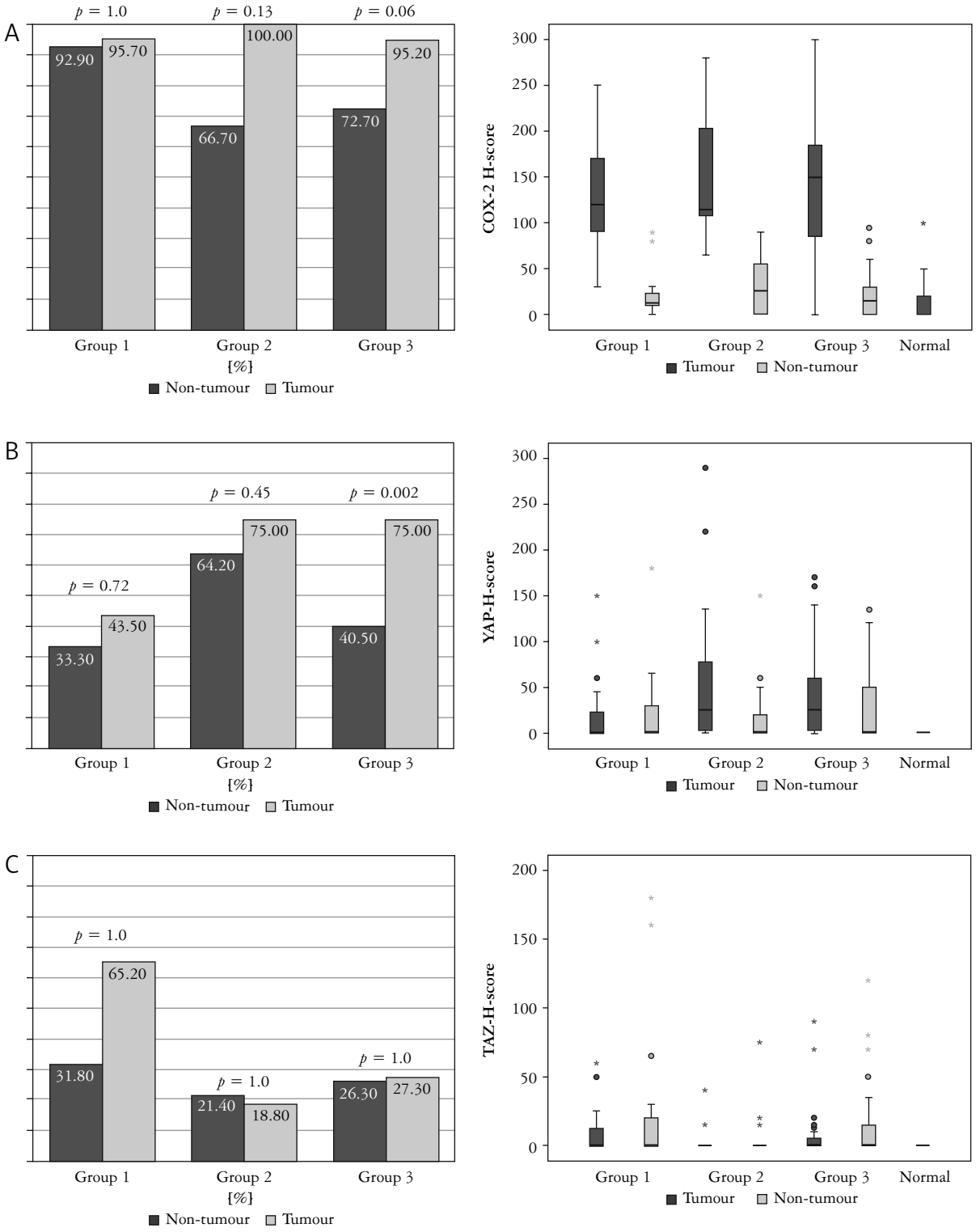

Fig. 2. Comparative expression of cyclo-oxygenase-2 (COX-2), yes-associated protein 1 (YAP), and transcriptional co-activator with PDZ-binding motif (TAZ) in normal, adjacent non-tumour, and tumour tissues in the 3 HCV-related hepatocellular carcinoma (HCC) groups. A) COX-2 expression was not significantly different between HCC and adjacent non-tumour tissue (left side); a box plot shows significant COX-2 overexpression in HCC compared with normal and adjacent non-tumour tissues (right side) in the 3 HCC groups. B) YAP was significantly overexpressed in HCC after direct-acting antiviral agent treatment compared with the corresponding non-tumour tissue (left side); a box plot shows significant overexpression of YAP in both HCC and adjacent non-tumour tissue compared with normal tissue (right side) in the 3 HCC groups. C) TAZ expression was not significantly different between HCC and adjacent non-tumour tissue (left side); a box plot shows significant overexpression of TAZ in HCC compared with normal tissue (right side) in the 3 HCC groups

Table II. Comparison between COX-2, YAP, and TAZ expressions in tumour, adjacent non-tumour, and normal liver tissue in 3 groups

Parameters	Tumour	Non tumour tissue	Control	*p*-value
		Group 1 (n = 23)		
COX-2 H-score				
Mean ±SD	128.41 ±60.14	22.86 ±27.42	17 ±33.35	$p^1 = 0.001$**
Median	120.00	12.50	0	$p^2 < 0.001$**
Min–max	30–250	0–90	0–100	$p^3 = 0.07$
YAP H-score				
Mean ±SD	20.00 ±38.02	20.48 ±42.03	0 ±0	$p^1 = 0.84$
Median	0.00	0.00	0.00	$p^2 = 0.02$*
Min–max	0–150	0–180	0–0	$p^3 = 0.04$*
TAZ H-score				
Mean ±SD	8.74 ±16.38	22.50 ±50.42	0 ±0	$p^1 = 0.44$
Median	0.00	0.00	0.00	$p^2 = 0.04$*
Min–max	0–60	0–80	0–0	$p^3 = 0.05$*
		Group 2 (n = 16)		
COX-2 H-score				
Mean ±SD	151.33 ±65.85	31.33 ±32.029	17 ±33.35	$p^1 = 0.002$**
Median	115.00	25.50	0	$p^2 < 0.001$**
Min–max	65–280	0–90	0–100	$p^3 = 0.15$
		Group 2 (n = 16)		
YAP H-score				
Mean ±SD	59.06 ±86.202	23.08 ±43.086	0 ±0	$p^1 = 0.09$
Median	25.00	0.00	0.00	$p^2 = .001$**
Min–max	0–290	0–150	0–0	$p^3 = 0.02$*
TAZ H-score				
Mean ±SD	4.38 ±10.78	7.86 ±20.354	0 ±0	$p^1 = 0.89$
Median	0.00	0.00	0.00	$p^2 = 0.15$
Min–max	0–40	0–75	0–0	$p^3 = 0.13$
		Group 3 (n = 44)		
COX-2 H-score				
Mean ±SD	137.38 ±73.28	20.52 ±23.10	17 ±33.35	$p^1 < 0.001$**
Median	150.00	15.00	0	$p^2 < 0.001$**
Min–max	0–300	0–95	0–100	$p^3 = 0.13$
YAP H-score				
Mean ±SD	40.91 ±46.80	24.86 ±40.147	0 ±0	$p^1 = 0.02$*
Median	25.00	0.00	0.00	$p^2 < 0.001$**
Min–max	0–170	0–135	0–0	$p^3 = 0.02$*
TAZ H-score				
Mean ±SD	8.14 ±21.42	14.47 ±29.35	0 ±0	$p^1 = 0.15$
Median	0.00	0.00	0.00	$p^2 = 0.06$
Min–max	0–90	0–120	0–0	$p^3 = 0.07$

*COX-2 – cyclo-oxygenase-2, H-score- histoscore, N – number of patients, p^1 – tumour and normal (Mann-Whitney U test, for continuous data) (χ^2, Exact for qualitative data), p^2 – tumour and adjacent (Wilcoxon test, for continuous data) (McNemar's test for qualitative data), p^3 – adjacent and normal (Mann-Whitney U test, for continuous data) (χ^2, Exact for qualitative data) SD – standard deviation, TAZ – transcriptional coactivator with PDZ-binding motif, YAP – yes-associated protein 1, * significant, ** highly significant*

Table III. Comparison between COX-2, YAP, TAZ expressions in three hepatocellular carcinoma groups

Parameters	No treatment $N = 23$	IFN $N = 16$	DAAs $N = 44$	Kruskal-Wallis *p*-value
COX-2 positive expression (%)	95.70	100.00	95.20	0.49
COX-2 H-score Mean ±SD	128.41 ±60.146	151.33 ±65.859	137.38 ±73.287	0.7
YAP positive expression (%)	43.50	75.00	75.00	0.02*
YAP H-score Mean ±SD	20.00 ±38.019	59.06 ±86.202	40.91 ±46.808	0.03*
TAZ positive expression (%)	65.20	18.80	27.30	0.54
TAZ H-score Mean ±SD	8.74 ±16.385	4.38 ±10.782	8.14 ±21.421	0.5

COX-2 – cyclo-oxygenase-2, DAAs – direct acting anti-viral agents, H-score – histoscore, IFN – interferon, N – number of patients, SD – standard deviation, TAZ – transcriptional co-activator with PDZ-binding motif, YAP – yes-associated protein 1

non-tumour liver tissue. This indicates that YAP/TAZ is overexpressed in the early stage of carcinogenesis and that the expression is maintained as HCC progresses. Moreover, high YAP/TAZ expression in non-tumour liver tissue adjacent to HCC may predict tumour relapse after successful surgical resection. Therefore, selective inhibitors of YAP/TAZ could play an important role not only in the treatment of HCC but also in primary prevention. However, the current study showed high expression of YAP in tissue from post-DAA-treated HCC compared with adjacent non-tumour liver tissue. This substantiates the carcinogenic effect of YAP and could be attributed to loss of cross-talk between YAP and the host immune response mediated by IFN after viral clearance [25]. This finding was in agreement with previous studies that reported significant YAP overexpression in HCC compared with adjacent non-tumour tissue [26, 27]. The limitations of those studies could be attributed to the small sample sizes (39 cases with no reported aetiological background) [26]. In a mouse model, YAP/TAZ expression was upregulated in peritumoral hepatocytes but not to the level seen in tumour tissue. However, the high peritumoral YAP/TAZ expression demonstrated an independent role in restraining tumour growth through inhibition of tumour cell proliferation [27]. Therefore, YAP/TAZ inhibition could produce undesirable protumourigenic effects. Therefore, additional studies are needed to elucidate the precise function of YAP/TAZ in HCC to determine whether these proteins are oncogenes or tumour suppressors.

The present study showed almost no significant association between COX-2 or YAP/TAZ expression and clinicopathological parameters of HCC cases. In addition, no impact of the expression of these markers on OS was observed. Previous studies on the prognostic role of COX-2 reported conflicting results; some studies found that its expression was correlated with a favourable prognosis, while others found that its expression was correlated with tumour aggressiveness [17, 28]. Similar conflicting data regarding YAP function in HCC, i.e. whether it functions as a tumour suppressor or an oncogene, have also been reported [29, 30]. The difference in the aetiology of HCC cases and in those treated with HCV regimens may implicate a prognostic effect. In addition, the different cut-off values and the scoring systems used in each study may have contributed to the observed heterogeneity [17].

In the current study, no significant association was found between COX-2 and YAP/TAZ expression in HCC cases. This could be explained by the reciprocal, regulatory feedback role of COX-2 and YAP/TAZ at a transcriptional level in human cell lines, which does not necessitate increased protein expression [10].

Although an insignificant relationship was observed between COX-2 and YAP/TAZ expression, their expression was upregulated during HCC development. Therefore, further analysis after subgrouping of HCC cases into YAP^+/TAZ^+ and YAP^-/TAZ^- was performed. In the YAP^+/TAZ^+ HCC group, COX-2 expression was associated with poor prognostic parameters including tumour multifocality, large tumour size, and advanced pathological stage. These findings suggest that YAP/TAZ expression may potentiate the poor prognostic role of COX-2, which fails to exacerbate malignant transformation with-

Table IV. The correlation of COX-2 expression with the clinicopathological parameters in YAP+/TAZ+ hepatocellular carcinoma cases

Parameters	YAP+/TAZ+ *n* = 16	Test	*p*-value
Age			
≤ 60	121.8 ±63.87	U = 41.5	0.97
> 60	150 ±00		
Sex			
Male	136.67 ±59.29	U = 0.00	0.11
Female			
Previous HCV treatment			
IFN	115 ±0	K = 2	0.16
DAAs	116.88 ±85.89		
No	147.5 ±23.27		
AFP [ng/ml]			
≤ 200	170.83 ±33.01	U = 5.5	0.045*
> 200	102.5 ±61.95		
Tumour focality			
Solitary	109 ±67.89	U = 3.5	0.051*
Multiple	183.33 ±28.87		
Tumour size			
≤ 5	78.33 ±70.19	U = 5.5	0.026*
> 5	167.14 ±31.07		
Pathological grade			
I	0 ±0	K = 2.59	0.27
II	132.78 ±67.64		
III	148.33 ±27.54		
Pathological stage			
Early stage	112.73 ±65.59	U = 000	0.029*
Late stage	200 ±0		
Lymph vascular invasion			
Negative	96 ±75.12	U = 10	0.14
Positive	145 ±60.89		
Recurrence			
Free	121.67 ±71.98	U = 14	0.54
Yes	136.25 ±67.99		
Non-tumour liver			
Cirrhosis	120.5 ±76.72	U = 15	1
Non-cirrhosis	145 ±27.84		

*AFP – α-fetoprotein, DAAs – direct acting anti-viral agents, IFN – interferon, K – Kruskal-Wallis, N – number, TAZ – transcriptional co-activator with PDZ-binding motif, U – Mann-Whitney U test, YAP – yes associated protein1, * significant*

out YAP/TAZ [31]. Xu *et al*. found that YAP played an important role in COX-2-induced carcinogenesis in HCC through activation of the Wnt/β-catenin pathway [11]. Cross-talk between COX-2 and the Wnt/β-catenin pathway has been reported to mediate tumour aggressiveness and metastasis in different cancers [32, 33]. Furthermore, in neurofibromatosis type 2 (NF2), YAP was found to promote the transcription of several targets, including prostaglandin-endoperoxide synthase 2 (PTGS2), which codes

for COX-2 and results in the overgrowth and survival of NF2-null Schwann cells [34]. In addition, PTGS2 (COX-2) has also been identified as a direct target of YAP in a pancreatic ductal adenocarcinoma model [35]. In urothelial carcinoma, the synergistic expression of YAP and COX-2 may indicate a more aggressive tumour phenotype and tumour stemness independent of other tumorigenic pathways [36].

Several studies have emphasized the potential role of COX-2 and/or YAP inhibitors in the prevention and treatment of several human cancers [37, 38]. Even the dual blockade of both pathways could improve chemo-responsiveness in urothelial cancer [36]. Sorafenib is a multikinase inhibitor approved for the treatment of advanced HCC that prolongs OS [39]. Sustained treatment with sorafenib could induce hypoxia through activation of hypoxia-inducible factor (HIF) synthesis, which leads to tumour angiogenesis [40]. Prolonged HIF activity could induce COX-2 and YAP activation and hamper the efficacy of sorafenib [41]. Therefore, concurrent targeting of COX-2/YAP with sorafenib therapy might improve the clinical management of patients with advanced HCC. Limitations of this study include a relatively small sample size, mainly in the YAP^+/TAZ^+ HCC subgroup. Although TMA immunohistochemistry has become an established technique in the assessment of protein expression in cancer, this technique still has some limitations in accuracy due to tumour heterogeneity.

Conclusions

No convincing difference was observed in COX-2 or YAP/TAZ expression between the untreated HCV group and both the IFN- and DAA-treated groups. Cyclo-oxygenase-2 may play a late role in the progression of HCC, while YAP/TAZ could play an early role in HCC progression. The poor prognostic role of COX-2 in HCC could be modulated by the combined expression of YAP/TAZ proteins.

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