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Molecular evaluation of apoptotic versus antiapoptotic angiogenic markers in hepatocellular carcinoma

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Abstract

Objective: To assess the role of HO-1 in HCC progression and to study the expression of apoptotic factors represented by TNF-α, and Fas-L versus antiapoptotic and angiogenic factors represented by HO-1, TGF-β, HGF, and VEGF in HCC compared to non cancerous cirrhotic liver.

Design and methods: Liver biopsies were taken from twelve patients with grade II HCC confined to the liver and twelve patients with non cancerous liver cirrhosis (served as control). RT-PCR of previous genes was evaluated.

Results: HO-1, VEGF, HGF, and TNF-α genes were significantly increased ($P<0.05$) in HCC compared to control. Fas-L showed a significant decrease ($P<0.05$) in HCC compared to control. TGF-β was higher in HCC than control but the difference was not statistically significant ($P>0.05$). HGF showed significant positive correlation with HO-1 ($r=0.8217$, $P=0.001$).

Conclusion: HCC is associated with increased expression of VEGF, HGF, and TGF-β, and with suppression of Fas-L. In addition, HO-1 is highly significantly expressed in HCC. The significant positive correlation between HO-1 and HGF was first reported in Egyptian human liver biopsies, and this suggests that it may play a role in the progression of hepatocellular carcinoma.

Keywords: Hepatocellular carcinoma; Heme oxygenase; Angiogenesis; Apoptosis

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant disorders, and in some parts of the world represents the most common neoplastic cause of death [1]. The majority of patients who have developed HCC in the low incidence geographic regions gave a history of cirrhosis, often as a sequel of hepatitis [2]. The mechanism of the progression from cirrhosis to HCC is still poorly understood.

Perturbation of hepatocyte growth regulation is mediated by a network of growth factors and cytokines that regulate the induction of hepatocyte proliferation and apoptosis [3]. Imbalance between apoptosis versus antiapoptotic factors is supposed to play important role in tumor progression.

Tumor associated angiogenesis is essential for tumor growth and metastasis and consists of multiple and sequential steps regulated by proangiogenic and angiogenic factors. Vascular endothelial cell proliferation is involved in this process with vascular endothelial growth factor (VEGF) as a proangiogenic molecule [4].

Heme oxygenase-1 (HO-1) is a stress response protein that may function as an antiapoptotic angiogenic factor that may have an important protective and beneficial effects for tumor cells growth in additional to its role against oxidative stress [5].

Transforming growth factor beta (TGF-β) is a multifunctional factor that induces a wide variety of cellular processes. It has been proved that TGF-β enhances β-integrin expression and consequently enhances cell adhesion and migration as previously detected in HCC cell lines [6].

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Table 1
Oligonucleotide primers sequences used for the different assays

Parameters	Primer sequence	PCR product (bp)
HO-1	sense 5′-CAGGCAGAGAATGCTGAGTTC-3′ antisense 5′-GCTTCACATAGCGCTGCA-3′	271
VEGF	sense 5′-ATGGCAGAAGGAGGGCAGCAT-3′ antisense5′-TTGGTGAGGTTTGATCCGCATCAT-3′	255
TGF-β	sense 5′-CTTCAGCTGCACAGAGAAGAACTGC-3′ antisense5′-CACGATCATGTTGGACAACTGCTCC-3′	298
TNF-α	sense 5′-AAAGCTTATGAGCACTGAAAGCATCAT-3′ antisense5′-ATGTAGATCACAGGGCAATGATCCCAAAG-3′	750
HGF	sense 5′-GCAATGTTGACTTCACTGAC-3′ antisense5′-GGTGGGAGAAACTAAAGTGC-3′	170
Fas-L	sense 5′-CAGTATCTTATGTAGAGCCC-3′ antisense5′-CCTGACAGTTCTAAGTCCAGG-3′	298
β-actin	sense 5′-TCACCCTGAAGTACCCCATGGAG-3′ antisense5′-TTGGCCTTGGGGTTCAGGGGG-3′	250

Hepatocyte growth factor (HGF) initially identified as "scatter factor" is produced by mesengial cells and exerts multiple biological activities such as morphogenesis, mitogensis and pro- or antiapoptotic effects on a variety of neoplastic epithelial cells [7]. HGF provides protection against H_2O_2-induced mesengial cells apoptosis through induction of HO-1 [8].

Tumor necrosis factor alpha (TNF-α) is a pleiotropic cytokine acting in paracrine or autocrine fashion on a wide variety of target cells via two members of the TNF-α receptor family, TNF receptor-1 (TNFR-1) and TNF receptor-2 (TNFR-2). Binding of TNF-α to these receptors activates signal transduction pathways that lead to different events that affects cell function and survival [9], including: (a) TNF-α supports the formation of pro-inflammatory molecules that are constituents of the immune response. (b) TNF-α can exert mitogenic actions and support proliferation processes. (c) TNF-α can induce apoptosis via caspase 8 activation and the mitochondrial pathway. (d) TNF-α supports survival pathway most probably via the formation of antiapoptotic proteins [9].

Fas and Fas-L are proteins related to tumor necrosis factor family of receptors and ligands [10]. The identification of Fas receptor (Fas, APO-I/CD95) and its ligand (Fas-L/CD95L) as a major regulator of both apoptosis and immune function has provided insight into an attracted mechanism of tumor escape for immune clearance [11].

The aim of this work was to assess the role of human heme oxygenase-1 in HCC progression and to study the expression of apoptotic factors represented by TNF-α and Fas-L versus anti-apoptotic and angiogenic factors represented by HO-1, TGF-β, VEGF, and HGF in HCC compared to non cancerous cirrhotic liver. We also aimed to find if there is a statistical correlation between HO-1 and the different studied parameters.

Materials and methods

Materials

Twenty four male patients were included in this study. They were selected from the Internal Medicine Outpatient Clinic at Kasr EL Eini Hospital, Cairo University. Their ages ranged from 35 to 55 years. Patients were classified into two groups.

Group I

Twelve male patients with grade II HCC confined to the liver with a mean age of (43.2 ± 6.01).

Group II

Twelve male patients with liver cirrhosis with a mean age of (41.8 ± 5.6) served as a control.

Methods

Liver biopsies were taken after a written consent from all patients who agreed to be members in the present study. The liver biopsies were immediately immersed in lysis buffer and stored at −80 °C till RNA extraction and polymerase chain reaction (PCR) were carried out.

RNA extraction

Total RNA was extracted from the liver according to the method of Chomkczynski and Sacch (1987) [12], using QI amp RNA kit provided by Qiagen Inc, USA. The extracted RNA was quantitated by spectrophotometry at 260 nm.

RT-PCR

RT-PCR experiments were done for detecting human HO-1, VEGF, Fas-L, TGF-β, TNF-α, and HGF using the corresponding primer sequences as shown in (Table 1). The different primers were prepared using the Oligo-1000 DNA synthesizer

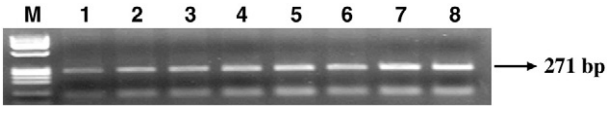

Fig. 1. Expression of HO-1 mRNA: RT-PCR products in liver cirrhosis and HCC. Lane M: Molecular DNA marker (Φ-x 174 Hae III). Lane 1: PCR product of HO-1 in liver cirrhosis. Lanes 2–8: PCR products of HO-1 in HCC.

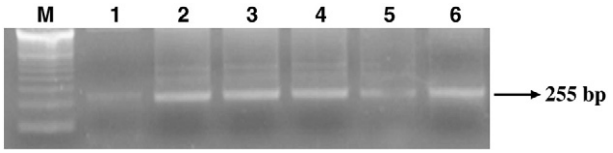

Fig. 2. Expression of VEGF mRNA: RT-PCR products in liver cirrhosis and HCC. Lane M: Molecular DNA marker (100 bp each). Lane 1: PCR product of VEGF in liver cirrhosis. Lanes 1–6: PCR products of VEGF in HCC.

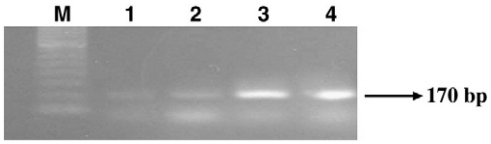

Fig. 4. Expression of HGF mRNA: RT-PCR products in liver cirrhosis and HCC. Lane M: Molecular DNA marker (100 bp each). Lane 1, 2: PCR product of HGF in liver cirrhosis. Lanes 3 and 4: PCR products of HGF in HCC.

(Beckman, California, USA) at the Unit of Biochemistry and Molecular Biology, Faculty of Medicine, Cairo University.

RT-PCR for heme oxygenase-1. 5 μg RNA was reverse transcribed using 12.5 μL $(dT)_{18}$ primer (final concentration 0.2 μM) and was denatured at 70 °C for 2 min. The denatured DNA was placed on ice and 6.5 μL of reverse transcription mixture containing 50 mM Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 0.5 mM of each dNTP's, 1 U/μL RNase inhibitor and 200 U MMLV reverse transcriptase was added. Then the reaction tube was placed at 42 °C for 1 h followed by heating to 92 °C to stop the reaction then placed on ice. The PCR rection was performed by adding to the reverse transcription tube, the PCR mix to a final volume of 100 μL. The PCR mix contained 10 mmol Tris–HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, and 0.001% gelatin, 250 μM dNTP's mix, 2.5 U Taq polymerase, and 100 μM of each primer. The reaction mixture was then subjected to 40 cycles of PCR amplification as follows: denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. After the last cycle, a final extension at 72 °C for 10 min was done. PCR products yielded a 271 bp on 1.5% agarose gel electrophoresis.

RT-PCR for VEGF. cDNA was generated with 1 μL (20 pmol) oligo $(dT)_{18}$ primer and 0.8 μL RNA reverse transcriptase for 60 min at 37 °C. For PCR, 4 μL cDNA were incubated with 30.5 μL water, 4 μL 25 mM $MgCl_2$, 1 μL dNTP's (10 mM), 5 μL 10× PCR buffer, 0.5 μL (2.5 U) Taq polymerase and 2.5 μL of each primer containing 10 pmol. A non selective primer sequence was used for all VEGF variants. The reaction mixture was subjected to 40 cycles of PCR amplification as follows: denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 2 min. The PCR product yielded a 255 bp fragment on 1.5% agarose gel electrophoresis.

RT-PCR for TGF-β. 5 μg of total RNA was reversed transcribed using RT-PCR Stratagene kit (Foster city, California, USA) according to the manufacturer's instructions. Then the reaction tube was placed at 42 °C for 1 h followed by heating to 95 °C for 5 min to inactivate the reverse transcriptase enzyme.

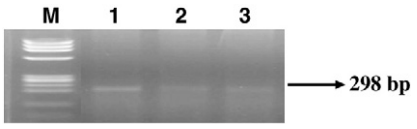

Fig. 3. Expression of TGF-β mRNA: RT-PCR products in liver cirrhosis and HCC. Lane M: Molecular DNA marker (Φ-x 174 Hae III). Lane 1: PCR product of TGF-β in HCC. Lanes 2 and 3: PCR products of TGF-β in liver cirrhosis.

For PCR, 3 μL cDNA was mixed with 5 μL reaction buffer (500 mmol/L KCl, 100 mmol/L Tris–HCl pH 8.3, 1.5 mmol/L $MgCl_2$, and 0.001% gelatin), 4 μL of 2.5 mmol/L dNTP's mix, 0.4 μL (2 U) Taq polymerase, 37.6 μL DEPC treated water and 1 μL of each primer containing 0.4 μmol/L. The reaction mix was then subjected to 35 cycles of PCR amplification as follows: denaturation at 94 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 2 min. The PCR product yielded 298 bp fragment on 1.5% agarose gel electrophoresis.

RT-PCR for Fas-L. Total RNA was reverse transcribed by oligo $(dT)_{18}$ primer, using reverse transcriptase and RNase inhibitor, then PCR was performed in a total volume 50 μL. The PCR mix was formed of PCR buffer, $MgCl_2$ (3 mmol), primers (30 pmol) each, dNTP's (200 μmol/L each) and Taq polymerase (2.5 U). The reaction mixture was then subjected to 40 cycles of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 2 min. The PCR product yielded a 298 bp fragment on 1.5% agarose gel electrophoresis.

RT-PCR for HGF. 5 μg of RNA was reverse transcribed into cDNA by adding the following: single strength RNA-PCR buffer, 5 mM $MgCl_2$, 1 mM dNTP mixture, 0.25 U/μL reverse transcriptase and 2.5 μM random primer. The mixture was incubated at 42 °C for 25 min, and then 5 min at 99 °C then chilled on ice. The PCR was performed in a total volume of 80 μL using the specific primers, Taq polymerase (2.5 U), $MgCl_2$ (4 mM). The PCR using primer pair specific for HGF was performed for 40 cycles, each cycle consists of a denaturation step at 94 °C for 1 min, an annealing step at 53 °C for 1 min, and an extension step at 72 °C for 2 min. The PCR product yielded a 170 bp fragment on 1.5% agarose gel electrophoresis.

RT-PCR for TNF-α. Reverse transcription was done using the RT-PCR kit (Promega, Madison, WI). Total RNA (1 μg), 6 μg dNTP's (10 mM), 6 μL 5× buffer, and 30 pmol specific downstream primer were bought up to a total volume of 28 μL. The mixture was heat shocked for 5 min at 65 °C, and chilled on ice for 5 min. Then 0.5 μL RNase inhibitor (40 U/μL) and 1 μL

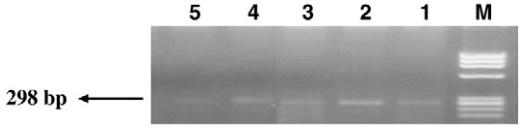

Fig. 5. Expression of Fas-L mRNA: RT-PCR products in liver cirrhosis and HCC. Lane M: Molecular DNA marker (Φ-x 174 Hae III). Lanes 1 and 2: PCR product of Fas-L in liver cirrhosis. Lanes 3–5: PCR products of Fas-L in HCC.

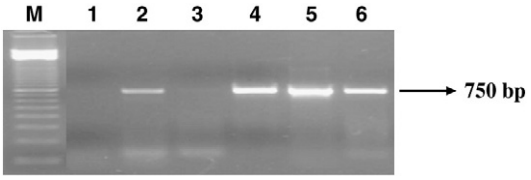

Fig. 6. Expression of TNF-α mRNA: RT-PCR products in HCC. Lane M: Molecular DNA marker (100 bp each). Lanes 1 and 3: PCR product of TNF-α in liver cirrhosis. Lanes 2, 4, 5, and 6: PCR product of TNF-α in HCC.

AMV (10 U/μL) were added and the mixture was incubated at 42 °C for 2 h. Then 5 μL of cDNA was added to the following PCR mixture: dNTP's (100 mM each), 10× PCR buffer containing $MgCl_2$, primers and Taq polymerase (2.5 U/μL). The PCR cycling condition was 30 cycles of 94 °C, 55 °C, 72 °C (each step 1 min). At the end an additional extension step for 7 min at 72 °C was performed. The PCR product yielded 750 bp fragments on 1.5% agarose gel electrophoresis.

RT-PCR for β-actin. The presence of RNA in all tissues was assessed by analysis of the "house-keeping" gene β-actin and to ensure equal loading volume of RNA. β-actin primers was shown in Table 1. β-actin, was amplified for 40 cycles, and the annealing temperature was 57 °C. The PCR product yielded 250 bp fragments on 1.5% agarose gel electrophoresis.

Agarose gel electophoresis

All PCR products were electrophoresed on 1.5% agarose gel with a size marker and were UV visualized by ethidium bromide staining.

Gel documentation

The PCR products were semiquantitated using the gel documentation system (Bio Doc Analyze) supplied by Biometra.

Results

Our results show no significant difference between both groups of patients as regard to the age and sex distribution.

Results are illustrated in Figs. 1–6 and Tables 2 and 3. HO-1expression was significantly higher ($P<0.05$) in HCC samples compared to liver cirrhosis (Fig. 1). The gene expression of VEGF was significantly increased ($P<0.001$) in HCC compared to liver cirrhosis (Fig. 2). In HCC the expression of TGF-β was higher in HCC compared to liver cirrhosis, but this increase was not statistically significant ($P<0.05$) (Fig. 3). The expression of HGF was significantly increased ($P<0.05$) in HCC compared to liver cirrhosis (Fig. 4). The expression of Fas-L was significantly decreased ($P<0.001$) in HCC compared to liver cirrhosis (Fig. 5). The expression of TNF-α was significantly increased ($P<0.05$) in HCC compared to liver cirrhosis (Fig. 6).

Our results also showed a positive correlation between HO-1 and HGF ($r=0.8217$, $P=0.001$) (Table 2).

Table 2
DNA concentration (μg/ml) of the PCR products of all studied genes in HCC and liver cirrhosis

Parameters	Liver cirrhosis	HCC
HO-1	255.23±111.34	2522.07±1348.57*
VEGF	175.86±77.2	2145.3±1265.82**
TGF-β	162.6±13.85	320.97±157.1
Fas-L	1619.5±334.4	443.8±178.55**
TNF-α	00.00±000	318.48±148.86*
HGF	460.16±136.09	1345.4±522.61*

* $P<0.05$: statistically significant versus liver cirrhosis.
** $P<0.001$: statistically significant versus liver cirrhosis.

Table 3
Laboratory data of HCC and liver cirrhosis patients

	N	Minimum	Maximum	Mean	SD
ALT	24	24	78	43.25	±14.04
AST	24	28	142	74.15	±28.69
Total bilirubin	24	0.8	3.2	2.163	±0.692
Direct bilirubin	24	0.5	1.8	1.189	±0.426
*Alk. Phosph.	24	115	682	175.20	±122.38
S-albumin	24	3	4	3.04	±0.27
PT	24	13	17	15.15	±1.10
PC	24	54	85	67.18	±7.07
$WBC\times10^3$	24	4	8	6.03	±1.43
$RBC\times10^6$	24	3.2	4.5	3.820	±0.418
HB (g/dL)	24	8	11	9.97	±0.89
$Platelet\times10^3$	24	70	130	94.70	±16.10
AFP (ng/mL)	24	9	435	118.85	±124.75

Abdominal ultrasonography was done for all the patients, it showed that

- All patients had liver cirrhosis child C.
  - 12 patients had hepatic focal lesion(s) (HFL), HFL (s) were in the right lobe in 8 patients (70%), in the Lt lobe in 2 patients (15%) and in both right and left lobes in 2 patients (15%).
- Size of the tumor:
  In 5 cases (25%) the tumor size was >5 cm in diameter and in 7 cases (75%) it was <5 cm in diameter.
- The spleen was enlarged in 10 patients (85%) and of average size in 2 patients (15%).
- Minimal pelvic ascites was found in 7 patients (35%).
- Histopathological examination of a needle biopsy from the tumor was done for all patients, the results were:
  - HCC grade I in 10 patients (85%).
  - HCC grade II in 2 patients (15%).

Statistical analysis

Data are expressed as mean±SD. Significance was assessed by student's *t*-test and the correlation coefficient (*r*). $P<0.05$ was considered to be statistically significant.

Discussion

Hepatocellular carcinoma is one of the most common worldwide tumors, arising on top of a background of chronic inflammatory disease [13].

Liver damage ranges from acute hepatitis to hepatocellular carcinoma through apoptosis, necrosis, inflammation, immune response, fibrosis, ischemia, altered gene expression and regeneration [14].

Reactive oxygen species (ROS) and nitrogen species play a crucial role in the induction and progression of liver diseases independent from its etiology. They are involved in the transcription and activation of a large series of cytokines and growth factors that in turn can contribute to further production of ROS and nitrogen species [14]. ROS generated by chronic inflammation are considered among the important factors that contribute to human hepatic carcinogenesis [15]. Furthermore, ROS have been reported to be among the powerful inducers of HO-1 enzyme [16]. In hepatocellular carcinomas, an inverse relationship was found between the expression of HO-1 and the degree of tumour differentiation, being negative in poorly differentiated tumours. In experimental model, down modulation of HO-1 expression correlated with malignancy progression. Thus, activation of HO-1 acts as a potential therapeutic tool [17].

In the present study HO-1 gene expression showed a significant quantitative increase ($P<0.05$) in cases of hepatocellular carcinoma compared to liver cirrhosis. Induction of HO-1 is thought to be of considerable importance in the initiation of cellular protective mechanisms following the exposure to various forms of stressful stimuli [18].

There are various possible mechanisms that may be responsible for the up regulation of HO-1, [19], reported a high expression of HO-1 in the liver of a rat model at the early stage of hepatoma. The inflammatory cytokines produced by the surrounding tissues at the hepatoma stage could be expected to play a role in the induction mechanism [20]. Also reported that cytokines are also potent inducers of HO-1. Tanaka et al., [5] reported that HO-1 may function in the antiapoptotic defense mechanism of the tumor and protects tumor cells against oxidative stress induced by nitric oxide (NO), which was found to be produced in excess during solid tumor growth in vivo.

Up regulation of HO-1 was observed with several angiogenic stimulatory factors such as tumor necrosis factor-α (TNF-α), interleukin-1 and 6 and TGF-β [21].

TNF-α is a proinflammatory cytokine produced in response to tissue injury, endotoxin exposure and infection [13]. Our results showed a significant quantitative increase ($P<0.05$) in TNF-α expression in HCC compared to liver cirrhosis. This can be explained by the study of Wheel house et al, [13] who reported that TNF-α causes formation of deoxyguanosine, an established marker of oxidative damage associated with chronic hepatitis in the human liver. The increase in the DNA damage did not result in p53 stabilization and TNF-α caused an increase in cell cycle progression. This indicates a possible role of TNF-α in the early stages of malignant transformation of hepatocytes. It is known that mitochondrial generation of ROS is involved in the cytotoxicity of TNF-α [22]. From the above, we conclude that TNF-α signaling in hepatocytes is associated with an increase in oxidative stress which may play a role in the induction of HO-1.

TGF-β is a regulatory cytokine that has been implicated in cell proliferation and migration. In a variety of diseases it promotes extracellular matrix deposition and proinflammatory events. TGF-β is involved in the regulation of liver cell proliferation and the escape of hepatoma cells from the growth restraining signals of TGF-β has been suggested to contribute to tumor development [23]. In early stages of hepatocarcinogenesis, IFN-alpha2b could enhance the proapoptotic effects of TGFbeta1, which could be highly beneficial in cancer therapy [24].

In our study, we found a quantitative increase in TGF-β expression in HCC compared to liver cirrhosis though this increase was not statistically significant ($P>0.05$).

These results are in agreement with those of Sacco et al. [25], who reported that the elevated serum TGF-β levels in HCC patients was greater than in liver cirrhosis patients.

The increase in TGF-β expression in our study may contribute to the up regulation of HO-1 in HCC. This is supported by the work of Kapturczak et al. [26], who reported that TGF-β via autocrine and paracrine pathways may induce HO-1 and serve as a protective response in injurious stimuli. Ning et al. [27] also reported that TGF-β stimulates HO-1 via the p38 mitogen activated protein kinase. On the other hand Abdel Aziz et al., [28], reported that HO-1 has an inhibitory effect on the expression of TGF-β. This may explain the non significant increase of TGF-β in HCC compared to liver cirrhosis.

The present study also revealed a relationship between HO-1 and apoptotic factors such as Fas-L. The quantitative expression of Fas-L showed a significant decrease ($P<0.001$) in HCC patients compared to liver cirrhosis patients. The decline in Fas-L expression may be due to HO-1 or other factors. Our results are supported by results of Ke et al. [29], who reported that HO-1 could be essential in preventing CD95/Fas-L-mediated apoptosis and prolong liver allograft survival via a downstream HO–CO signaling pathway.

Tumor angiogenesis is critical to both the growth and metastasis of cancer, and is regulated by angiogenic factors [30]. It has been proved that HO-1 gene has responsive elements for many angiogenic agents and induces marked neovascularization [31,32].

Vascular endothelial growth factor (VEGF) is closely related to angiogenesis in various human cancers and seems to play an important role in tumor progression [33]. Moreover, it could be used as an indicator for tumor angiogenesis [34]. VEGF appears to be involved in the development of HCC and it could be a predictor of HCC development in patients with cirrhosis [35].

Our results showed a significant quantitative increase ($P<0.001$) in the expression of VEGF in HCC compared to liver cirrhosis. The increased expression of VEGF in HCC could be explained by several mechanisms. The work of Abdel Aziz et al. [28], reported that HO-1 has a stimulatory effect on VEGF expression and that the induction of VEGF is a possible mechanism in HO-1 mediated angiogenesis. This was further documented by Dulak et al., [36], who reported that HO-1 is induced by ROS and NO and the genetic over expression of HO-1 enhanced VEGF synthesis and augmented formation of vascular capillaries. Furthermore, distortion of autocrine TGF-β signals in HCC accelerates their malignant potential by cell

growth as well as plasminogen activator–inhibitor type1 (PAI-1) and VEGF production [37].

In addition, HGF promotes hepatocarcinogenesis through the stimulation of angiogenesis by HGF itself and/or indirectly through VEGF [38]. In our work, HGF was highly expressed in HCC ($P<0.05$) compared to liver cirrhosis. HGF is a multifunctional cytokine of mesenchymal origin [39]. In the liver HGF is produced by nonparenchymal cells and affect growth and proliferation of parenchymal cells [40]. During liver regeneration HGF gene expression in the liver was reported to be increased [41]. Miura et al. [42] also reported that ROS induces HGF gene expression in mesothelial cells.The Akt/PI-3 kinase pathway is a system essential for cell survival and was found to promote cell survival in hepatocyte-derived cell lines (HCC, hepatoblastoma, and embryonic hepatocytes). Yang et al. 2007 [43], reported that mRNA level of HGF is markedly increased in HCC. It protects cells from Fas-mediated cell death via Fas-death-inducing signaling complex (DISC) formation as a result of Akt activation [44]. This may contribute to the decreased expression of Fas-L.

Our results showed that there is a positive correlation between HO-1 and HGF ($r=0.8217$, $P=0.001$) which was first reported for the first time in human HCC biopsy samples. Tacchini and coworkers 2001 [37], found a time dependent induction of HO-1 mRNA with the maximum (3–4 fold) 4 h after HGF treatment of HepG2 hepatoma cell line. Also Arakaki et al. [45] reported that cell treatment with HGF was associated with the generation of ROS. ROS are well known a potent inducer of HO-1. The correlation between HO-1 and HGF may play an important role in tumor progression as HGF induces the expression of HO-1. Both HO-1 and HGF promote the induction of VEGF. VEGF induces disruption of tight junctions in PKC-alpha dependent manner. In addition to its known angiogenic properties, VEGF may promote HCC spreading into normal liver parenchyma [46].

So we can conclude that HCC is associated with accelerated markers of angiogenesis and antiapoptotic factors represented by HO-1, VEGF, HGF, TNF-α and TGF-β and suppression of markers of apoptosis represented by Fas-L. HO-1 may upregulate HGF gene expression and may function as an antiapoptotic defense of the tumor against oxidative stress.

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