The Expression of PEDF and its Putative Receptors in Hepatocellular Carcinoma and Background Liver Tissue

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Abstract. *Background/Aim:* Hepatocellular carcinoma (HCC) remains one of the biggest medical issues. Pigment epithelial-derived factor (PEDF) is a glycoprotein that belongs to the superfamily of serine protease inhibitors. PEDF interacts with its two receptors, adipose triglyceride lipase (ATGL) and laminin receptor (LR). *Materials and Methods:* We conducted immunohistochemical staining for PEDF, LR and ATGL in 151 resected HCCs and their background liver tissues. *Results:* High expression of LR in HCC was associated with high histological grade and portal vein invasion, while high expression of PEDF in HCC was associated with absence of portal vein invasion. High LR expression in background liver was statistically associated with low serum albumin levels and was an independent prognostic factor of worse outcomes. No cases with more than 5% fatty degeneration in the background liver tissue showed high PEDF expression. *Conclusion:* PEDF/LR/ATGL could be potential biomarkers in HCC and various chronic hepatic disorders.

Hepatocellular carcinoma (HCC) is the sixth most common cancer and the fourth leading cause of cancer-related mortality worldwide. The hepatitis B virus (HBV), hepatitis C virus (HCV), alcoholic intake, and non-alcoholic steatohepatitis are known to be major causes of hepatocarcinogenesis. In developed countries, anti-viral treatments for HBV and HCV facilitate the decrease in viral related HCC development; however, the incidence of alcohol and non-alcoholic steatohepatitis-related HCC is increasing (1). Additionally, approximately three quarters of all new cases occur in low- and middle-income countries (2). Therefore, HCC remains a significant medical issue.

Pigment epithelial-derived factor (PEDF) is a glycoprotein that belongs to the superfamily of serine protease inhibitors. PEDF was first purified from the conditioned media of human retinal pigment epithelial cells and is found in various tissues, including adipocytes, vascular and inflammatory cells (3). PEDF is a potent endogenous inhibitor of angiogenesis (4).

We have previously reported that PEDF could alleviate the development and progression of steatohepatitis through the suppression of steatosis and inflammatory response in methionine- and choline-deficient diet-fed mice (5). PEDF has been found in various carcinomas, including HCC (6, 7), pancreatic carcinoma (8), melanoma (9), and ovarian cancer (10). Generally, PEDF has been linked to decreased metastases and a favorable prognosis in many human cancers (11).

PEDF interacts with its two putative receptors, adipose triglyceride lipase (ATGL) and laminin receptor (LR). ATGL is required for PEDF-induced lipolysis and triglyceride degradation

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in liver cells and adipocytes (12, 13), whereas LR mediates the anti-angiogenic activity of PEDF in endothelial cells (14). ATGL enhances the growth and motility of tumor cells (15). The expression of LR is increased in neoplastic cells compared to that in their normal counterparts, which directly correlates with an enhanced invasive and metastatic potential (16).

PEDF and its receptors seem to play an important role in several malignancies. However, the role of PEDF in HCC is debatable and the number of reports on this subject is limited (6, 7, 17). Therefore, to clarify the role of PEDF and its receptors, LR and ATGL, in the human liver, we performed immunohistochemistry (IHC) staining in 151 human liver samples, including HCC and its adjacent background liver.

Materials and Methods

Patients. One hundred fifty-one cases of previously diagnosed HCC were enrolled in this study. All patients had undergone resection at Kurume University Hospital between 2007 and 2011. Patients who underwent previous treatment for HCC, those without post-operative follow-up data, those who undertook non-curative surgical treatment, and those with autoimmune hepatitis or primary biliary cholangitis were excluded. The clinicopathological characteristics of the enrolled patients are described in Table I. Clinical follow-up data revealed a mean disease-free survival (DFS) of 921 (range=13-2657 days) and overall survival (OS) of 2149 (range=82-4346 days). This study was approved by the ethics committee of Kurume University (approval #19097).

Pathological assessment. For pathological diagnosis, we used the general rules for the clinical and pathological study of primary liver cancer edited by the Liver Cancer Study Group of Japan (18). Liver specimens were fixed in 10% formalin and embedded in paraffin. Consecutive 4- μ m sections were stained with hematoxylin and eosin. When a tumor nodule consisted of plural histological grades, the highest grade was adopted. All slides were evaluated by two certificated pathologists (J.A. and O.N.).

Tissue microarray preparation. Tissue microarrays (TMA) were built using an arraying instrument (Azumaya, Tokyo, JAPAN) to assemble tissue samples on a block. For each case, 4-mm core diameter samples were obtained from the tumor and background liver tissue from each "donor" block and placed on a separate "recipient" TMA block. Each slide contained 22 cores. We punctured paraffin-embedded samples in two cores from the tumor portion and one core from the background liver as well as four control tissues, which were obtained from an autopsy case and comprised the kidney, spleen, brain, and heart.

Immunohistochemical stain. We performed IHC staining on paraffin-embedded TMA sections using the following antibodies: PEDF (H-125, Santa Cruz Biotechnology, Dallas, TX, USA), laminin R (H-2, Santa Cruz Biotechnology), and ATGL (Cell Signaling Technology, Denver, MA, USA). IHC staining was performed using BOND-III (Leica Microsystems, Newcastle, UK). Tubular epithelium of the kidney was used as a positive control of PEDF and ATGL. Bile duct epithelium of the background liver was used as a positive control for LR. Each marker was evaluated according to its intensity and was scored from 0

Table I. Clinicopathological characteristics of 151 HCCs.

Gender	
Male	111
Female	40
Serum albumin (g/dl)	
<4.0	81
4.0 and more	70
Etiology	
HBs-Ag (+)	24
HCV-Ab (+)	86
HBs-Ag (+) and HCV-Ab (+)	3
HBs-Ag (-) and HCV-Ab (-)	38
Tumor size (mm)	
<20	42
≥ 20	109
Macroscopic type	
SNIM and SN	84
Others	67
Histological differentiation	
Well	12
Moderately	113
Poorly	26
Portal vein invasion	
Absent	70
Present	81
Intrahepatic metastasis	
Absent	130
Present	21
Background liver	
NL or CH	95
LC	56
Child-Pugh score	
A	144
B	7
Post-operative observation	
period (days: median)	
DFS	921 (13-2,657)
OS	2149 (82-4,346)

SNIM: Small nodular type with indistinct margin; SN: simple nodular type; NL: normal liver; CH: chronic hepatitis; LC: liver cirrhosis; DFS: disease-free survival; OS: overall survival.

to 3, regardless of staining proportion. A score of 3 indicated high expression, which was defined as an intensity equivalent to that of the positive controls. Scores of 2, 1, and 0 indicated low expression, which was defined as cases with positive signal but with lesser intensity than that indicated by score 3, cases with subtle intensity, and cases without any intensity, respectively. For the ATGL assessment of the background liver, score 2-1 and score 0 were regarded as high expression and low expression, respectively, due to the lack of cases of ATGL expression with a score of 3. Two authors (J.A. and T.Y.) independently conducted the immunohistochemical evaluation. Any disagreement between the pathologists was resolved by a joint review to obtain a single consensus.

Statistical analysis. Differences between groups were examined using the Chi-square test or the Fisher's exact test for categorical variables. Survival functions were estimated according to the

Figure 1. Immunohistochemical stain score distribution for PEDF, LR, and ATGL. The distribution of the immunohistochemical stain score for PEDF (A) and ATGL (C) was not statistically different, but that of LR (B) was significantly different. There were significantly more cases with an LR score of 3 in the HCC than in the background liver ($p=0.0031$).

Kaplan–Meier method. Differences between groups were analyzed using the log-rank test. The Cox proportional hazard model was applied to evaluate the effects of clinicopathologic factors while adjusting for potential confounding factors. As possible explanatory variables, all clinicopathologic factors with a p -value less than 0.05 in univariate analyses were included in the model. p -Values <0.05 were regarded as significant. All analyses were conducted using JMP version 14.2 software (SAS Institute Inc., Cary, NC, USA).

Results

IHC stain score distribution of PEDF, LR, and ATGL. The immunohistochemical stain score distribution of PEDF and ATGL was not statistically different between HCC and its background liver, but there was a significant difference in LR expression between HCC and the background liver (Figure 1). There were significantly more cases of LR expression with a score of 3 in HCC than in the background liver ($p=0.0031$, Figure 1). Representative microphotographs of high and low expression of PEDF, LR, and ATGL in HCC and the background liver are shown in Figure 2.

Expression of PEDF, LR, and ATGL in HCC. Portal vein invasion was significantly less frequent in patients with a high expression of PEDF ($p=0.0496$, Table II). Poorly differentiated HCC and portal vein invasion were significantly more frequent in those with high expression of LR ($p=0.0037$ and $p=0.0438$, respectively, Table II). No statistically significant relationship was found between ATGL expression and any clinicopathologic factors. There were no apparent relationships between the expression of laminin R, PEDF and ATGL, and patient prognosis, including DFS and OS in the log-rank test (Figure 3).

Expression of PEDF, LR, and ATGL in background non-neoplastic liver. In the background liver, there were significantly more cases with high PEDF expression in non-hepatitis B virus and non-hepatitis C virus (NBNC) ($p=0.0229$, Table III). No cases with more than 5% fatty degeneration showed high PEDF expression ($p=0.0002$, Table III). Cases with high LR expression in the background liver tissue showed a significantly low serum albumin levels, but were not associated with background liver conditions, such as chronic hepatitis and cirrhosis. No significant association was observed in clinicopathological factors, such as background liver conditions and serum albumin levels in ATGL. Furthermore, there was no apparent relationship between the Child–Pugh score and the expression levels of PEDF, LR, or ATGL. The expression of PEDF and ATGL in the background liver was not associated with prognosis (Figure 4). Cases with a high expression of LR showed a significantly worse prognosis in DFS and OS ($p=0.018$ and $p=0.030$, respectively, Figure 4).

Univariate and multivariate analysis for prognosis. In the univariate analysis for DFS, low serum albumin levels ($p=0.001$), large tumor size ($p=0.048$), intrahepatic metastasis ($p<0.001$), portal vein invasion ($p=0.002$), and high LR expression in the background liver ($p=0.020$) were significantly associated with a worse prognosis. Of these, low serum albumin levels and high LR expression in the background liver remained significant for DFS in multivariate analysis ($p=0.018$ and $p=0.021$, respectively) (Table IV).

In univariate analysis of OS, low serum albumin levels ($p=0.029$), intrahepatic metastasis ($p=0.017$), portal vein invasion ($p=0.027$), and high LR expression in the background liver ($p=0.035$) were statistically associated with

Figure 2. Representative microphotographs of high and low expression of PEDF, LR, and ATGL in HCC and background liver. High expression of PEDF in HCC (A, B). Low expression of PEDF in HCC (C, D). High expression of PEDF in the background liver (E, F). Low expression of PEDF in the background liver (G, H). Liver with fatty changes (G, H). High expression of LR in HCC (I, J). Low expression of LR in HCC (K, L). High expression of PEDF in the background liver (M, N). Low expression of PEDF in the background liver (O, P). High expression of ATGL in HCC (Q, R). Low expression of ATGL in HCC (S, T). High expression of ATGL in the background liver (U, V). Low expression of ATGL in the background liver (W, X).

worse prognosis. Among these variables, only high LR expression in the background liver remained significant for OS in the multivariate analysis ($p=0.030$) (Table V).

Discussion

This study revealed that high expression of LR in HCC was associated with an aggressive clinicopathological phenotype, while high expression of PEDF in HCC was associated with less aggressive properties. High LR expression in the background liver was statistically associated with a low

serum albumin levels and an unfavorable postoperative prognosis. PEDF suppressed fat degeneration in background liver tissue.

In this study, the PEDF expression levels in HCC were comparable with that in the background liver. Although the number of cases were limited, HCC cases with portal vein invasion and high PEDF expression were significantly less frequent than those with low PEDF expression. These findings indicate that the PEDF in HCC could be associated with less aggressive behavior. However, the role of PEDF in HCC is contentious. Kawaguchi *et al.*, reported that PEDF

Table II. Correlation between PEDF, LR and ATGL expression in hepatocellular carcinoma and various clinicopathological factors.

	PEDF			LR			ATGL		
	Low	High	<i>p</i> -Value	Low	High	<i>p</i> -Value	Low	High	<i>p</i> -Value
Tumor size			<i>p</i> =0.6738			<i>p</i> =0.1838			<i>p</i> =0.2854
>20 mm	41	1		10	32		21	21	
<20 or 20 mm	103	6		38	71		65	44	
Macroscopic type			<i>p</i> =0.4635			<i>p</i> =0.1283			<i>p</i> =0.0878
SNIM or SN	79	5		31	53		53	31	
Others	65	2		17	50		33	34	
Histological grade			<i>p</i> =0.4971			<i>p</i>=0.0037			<i>p</i> =0.1120
Well diff.	12	0		6	6		7	5	
Moderately diff.	106	7		40	73		69	44	
Poorly diff.	26	0		2	24		10	16	
Portal vein invasion			<i>p</i>=0.0496			<i>p</i>=0.0438			<i>p</i> =0.3013
Absence	64	6		28	42		43	27	
Presence	80	1		20	61		43	38	
Intrahepatic metastasis			<i>p</i> =0.2514			<i>p</i> =0.2502			<i>p</i> =0.6198
Absence	125	5		39	91		73	57	
Presence	19	2		9	12		13	8	
Fatty change in HCC			<i>p</i> =1.0000			<i>p</i> =0.8200			<i>p</i> =0.8479
<5%	121	6		36	79		65	50	
>5 or 5%	23	1		12	24		21	15	
Background liver tissue			<i>p</i> =0.7107			<i>p</i> =0.6652			<i>p</i> =0.3254
NL or CH	91	4		29	66		57	38	
LC	53	3		19	37		29	27	
Child-Pugh classification			<i>p</i> =0.5503			<i>p</i> =0.8515			<i>p</i> =0.9917
A	137	7		46	98		82	62	
B	7	0		2	5		4	3	

SNIM: Small nodular type with indistinct margin; SN: simple nodular type; diff: differentiation; NL: normal liver; CH: chronic hepatitis; LC: liver cirrhosis; PEDF: pigment epithelium derived factor; LR: laminin receptor; ATGL: adipose triglyceride lipase. Bold values indicate statistical significance.

expression in HCC was higher than that in background liver tissues; moreover, increased expression of PEDF may exert anti-apoptotic effects in HCC (7). Furthermore, Hou *et al.*, found that a high expression of PEDF, determined by IHC, was associated with a shorter overall survival of 88 HCC patients (6). In contrast, P18 peptide, a functional fragment of PEDF, has been reported to inhibit angiogenesis and tumor growth in HCC *via* the modulation of vascular endothelial growth factor (VEGF)/VEGF receptor 2 signaling pathway (17). Gao *et al.*, showed that intravenous injection of PEDF-expressing human mesenchymal stem cells significantly suppressed both the growth of primary liver tumors and the development of pulmonary metastases (19). Very recently, Li C *et al.*, proposed novel functions of PEDF in the liver (20). They reported that intracellular PEDF led to an accumulation of free fatty acids (FFAs) and eventually promoted HCC cell growth. Conversely, secreted PEDF acted as an anti-angiogenetic factor that inhibited tumor angiogenesis in HCC. Some reports have documented that PEDF could induce the differentiation of progenitor cells and that PEDF promotes Wnt/ β -catenin signaling in stem

cell populations but inhibits Wnt signaling in differentiated cells (21-23). Moreover, liver specific gene expression profiles of genetic and chemical perturbation of PEDF in knockout mouse liver were quite similar to a human HCC classification, Hoshida's liver cancer subclass S1, which showed markedly overactive Wnt/ β -catenin signaling (23, 24). Collectively, the expression and function of PEDF in HCC may not be universal, indicating that the effect of PEDF changes depend on the phase of HCC progression and/or HCC gene mutations.

Previous studies have demonstrated that PEDF, which is thought to have ATGL-binding ability, mediates ATGL degradation (12, 25). Li *et al.*, revealed that PEDF exacerbated the lipogenesis pathway, while impairing the FFAs oxidation pathway, leading to enhanced lipid accumulation in HCC cells (20). However, in our study, there was no apparent association between PEDF, ATGL, and fatty changes in HCC. In the background liver, the number of cases with high PEDF expression was significantly higher in the NBNC group. Interestingly, out of 23 cases with more than 5% fat degeneration in the background liver, no case

Figure 3. Correlation between patient prognosis and expression levels of PEDF, LR, and ATGL in HCC. There were no apparent relationships between the expression of PEDF (A, B), LR (C, D), and ATGL (E, F) in HCC and patient prognosis, including DFS and OS, respectively, in the log-rank test.

Figure 4. Correlation between patient prognosis and expression levels of PEDF, LR, and ATGL in background liver tissue. The expression of PEDF and ATGL in background liver was not associated with DFS and OS (A, B and E, F, respectively). Cases with a high expression of LR showed significantly worse prognosis in DFS (C) and OS (D) ($p=0.018$ and $p=0.030$, respectively).

Table III. Correlation between PEDF, LR and ATGL expression in background liver and various clinicopathological factors.

	PEDF			LR			ATGL		
	Low	High	<i>p</i> -Value	Low	High	<i>p</i> -Value	Low	High	<i>p</i> -Value
Fibrosis			<i>p</i> =0.8031			<i>p</i> =0.9672			<i>p</i> =0.9576
NL or CH	80	15		58	37		76	19	
LC	48	8		34	22		45	11	
Etiology			<i>p</i>=0.0229			<i>p</i> =0.3125			<i>p</i> =0.2522
HBV	18	6		17	7		17	7	
HCV	79	7		47	39		73	13	
NBNC	28	10		26	12		28	10	
HBV+HCV	3	0		2	1		3	0	
Serum albumin level			<i>p</i> =0.2889			<i>p</i>=0.0328			<i>p</i> =0.7102
>4 g/dl	71	10		43	38		64	17	
<4 or 4 g/dl	57	13		49	21		57	13	
Fatty change in background liver			<i>p</i>=0.0002			<i>p</i> =0.1057			<i>p</i> =0.5755
<5%	92	23		66	49		91	24	
>5 or 5%	36	0		26	10		30	6	
Child-Pugh classification			<i>p</i> =0.2508			<i>p</i> =0.8336			<i>p</i> =0.7047
A	121	23		88	56		115	6	
B	7	0		4	3		29	1	

NL: Normal liver; CH: chronic hepatitis; LC: liver cirrhosis; HBV: hepatitis virus, type B; HCV: hepatitis virus, type C; NBNC: non hepatitis virus, type B and non hepatitis virus, type C. Bold values indicate statistical significance.

Table IV. Cox regression analysis of univariate and multivariate analysis for disease-free survival.

Parameter		Univariate analysis		Multivariate analysis	
		HR (95%CI)	<i>p</i> -Value	HR (95%CI)	<i>p</i> -Value
Gender	Female/Male	0.769 (0.436-1.358)	0.366		
Albumin (g/dl)	≥4/<4	0.410 (0.241-0.698)	0.001	0.516 (0.298-0.894)	0.018
Background liver	LC/CH	1.302 (0.794-2.135)	0.295		
Tumor size (mm)	≥20/<20	1.852 (1.006-3.408)	0.048	1.656 (0.873-3.143)	0.123
Histological grade	Mode-por/well	1.033 (0.445-2.399)	0.939		
Macroscopic findings	Others/SN-SNIM	1.085 (0.660-1.782)	0.748		
Intrahepatic metastasis	Present/Absent	3.528 (1.928-6.454)	<0.001	2.640 (1.364-5.109)	0.250
Portal vein invasion	Present/Absent	2.235 (1.331-3.754)	0.002	1.601 (0.911-2.816)	0.102
Child-Pugh classification	B/A	1.684 (0.670-4.230)	0.268		
Fatty change in background liver	≥5%/<5%	0.806 (0.438-1.481)	0.487		
PEDF in HCC	High/Low	0.610 (0.149-2.495)	0.491		
LR in HCC	High/Low	0.745 (0.450-1.231)	0.250		
ATGL in HCC	High/Low	0.772 (0.465-1.282)	0.317		
PEDF in background liver	High/Low	0.744 (0.339-1.633)	0.461		
LR in background liver	High/Low	1.801 (1.098-2.956)	0.020	1.820 (1.093-3.032)	0.021
ATGL in background liver	High/Low	0.970 (0.506-1.857)	0.926		

LC: Liver cirrhosis; CH: chronic hepatitis; NL: normal liver; Others included simple nodular type with extranodular growth, confluent multinodular type and infiltrative type; SN: simple nodular type; SNIM: small nodular type with indistinct margin; PEDF: pigment epithelium derived factor; LR: laminin receptor; ATGL: adipose triglyceride lipase. Bold values indicate statistical significance.

showed high PEDF expression. These findings strongly support our previous study, which showed that PEDF could alleviate the development and progression of steatohepatitis through the suppression of steatosis in methionine- and

choline-deficient diet-fed mice (5). In that study, although no significant association between ATGL and fatty change was found, PEDF was shown to suppress fatty degeneration of the human liver tissue as well as in a mouse model.

Table V. Cox regression analysis of univariate and multivariate analysis for overall survival.

Parameter		Univariate analysis		Multivariate analysis	
		HR (95% CI)	p-Value	HR (95% CI)	p-Value
Gender	Female/Male	0.350 (0.105-1.165)	0.087		
Albumin (g/dl)	≥4/<4	0.380 (0.160-0.905)	0.029	0.539 (0.220-1.316)	0.175
Background liver	LC/CH-NL	1.525 (0.705-3.298)	0.284		
Tumor size (mm)	≥20/<20	3.079 (0.924-10.25)	0.067	2.404 (0.685-8.434)	0.171
Histological grade	Mode-por/well	2.446 (0.331-18.05)	0.380		
Macroscopic findings	Others/SN-SNIM	1.167 (0.540-2.523)	0.695		
Intrahepatic metastasis	Present/Absent	2.880 (1.209-6.859)	0.017	1.745 (0.676-4.502)	0.250
Portal vein invasion	Present/Absent	2.666 (1.119-6.350)	0.027	1.886 (0.731-4.867)	0.189
Child-Pugh classification	B/A	2.781 (0.834-9.268)	0.096		
Fatty change in background liver	≥5%/<5%	0.920 (0.369-2.293)	0.858		
PEDF in HCC	High/Low	1.922 (0.453-8.146)	0.375		
LR in HCC	High/Low	0.703 (0.319-1.549)	0.382		
ATGL in HCC	High/Low	0.504 (0.212-1.199)	0.121		
PEDF in background liver	High/Low	1.526 (0.575-4.048)	0.396		
LR in background liver	High/Low	2.299 (1.060-4.985)	0.035	2.395 (1.090-5.263)	0.030
ATGL in background liver	High/Low	1.432 (0.602-3.407)	0.417		

LC: Liver cirrhosis, CH: chronic hepatitis, NL: normal liver, Others included simple nodular type with extranodular growth, confluent multinodular type and infiltrative type, SN: simple nodular type, SNIM: small nodular type with indistinct margin, PEDF: pigment epithelium derived factor, LR: laminin receptor, ATGL: adipose triglyceride lipase. Bold values indicate statistical significance.

LR is one of putative receptors of PEDF. A previous study has revealed that PEDF is mainly bound to LR in HCC cells and tissues (6). That study revealed that high expression of LR as well as a high expression of PEDF could predict poor prognosis of HCC. In our study, high LR expression in HCC was not indicative of the patient's prognosis after operation. However, high LR expression was significantly associated with high histological grade and the presence of portal vein invasion, which are indicators of aggressive phenotypes of HCCs (26). Surprisingly, high LR expression in the background liver was significantly associated with poor prognosis, including DFS and OS. High LR expression in the background liver was an independent prognostic factor and was significantly correlated with lower serum albumin levels; however, it is not associated with the background liver condition and etiology.

Liver fibrosis is caused by an excessive accumulation of extracellular matrix (ECM), such as collagen type IV and laminin, due to chronic injury, which can progress to cirrhosis (27-29). When the laminin/laminin receptor signal becomes dominant compared to other ECMs, albumin production in hepatocytes is known to be decreased (30).

The serum albumin level is widely known to be one of the sensitive markers of liver preserve condition and is associated with prognosis (31, 32). indicating that LR expression could be enhanced due to chronic liver injury in advance of pathological recognition of apparent morphological changes. In our study, the expression of PEDF in the background liver did not correlate with that of LR.

PEDF expression was reduced in human cirrhosis, and PEDF restoration ameliorated fibrotic changes in some different experimental models (23, 33, 34).

Our present study has some limitations. We scrutinized the expressions of PEDF, LR, and ATGL in HCC and background liver tissues; however, we did not examine the functional aspects of these markers. The roles of these factors may be different between HCC and background liver tissues. It might be important to clarify the interactions between HCC and background liver tissues. Further investigations are required to validate our results.

In conclusion, we demonstrated the significance of PEDF and its receptors, LR and ATGL, in HCC and background liver tissue. PEDF/LR/ATGL could be potential biomarkers, especially of clinical prognosis, and provide therapeutic targets for both HCC and various chronic hepatic disorders.

Conflicts of Interest

The Authors state that there are no conflicts of interest to disclose in relation to this study.

Authors' Contributions

JA and TY designed this study and drafted the manuscript. JA and KM conducted immunohistochemical stain. TM, SIY and HY edited the manuscript. HK, YM, SM, YK, YN and ON participated in pathological diagnosis. TH, HS and KO acquired and collected the samples. ES performed the statistical analysis and drew the essential diagrams.

References

- Tateishi R, Uchino K, Fujiwara N, Takehara T, Okanou T, Seike M, Yoshiji H, Yatsuhashi H, Shimizu M, Torimura T, Moriyama M, Sakaida I, Okada H, Chiba T, Chuma M, Nakao K, Isomoto H, Sasaki Y, Kaneko S, Masaki T, Chayama K and Koike K: A nationwide survey on non-B, non-C hepatocellular carcinoma in Japan: 2011-2015 update. *J Gastroenterol* 54(4): 367-376, 2019. PMID: 30498904. DOI: 10.1007/s00535-018-1532-5
- Chapter 8, Tumours of the liver and intrahepatic bile ducts. *In*: WHO Classification of Tumours. 5th ed. Lyon: International agency for Research on Cancer, 2019.
- Tombran-Tink J, Chader GG and Johnson LV: PEDF: A pigment epithelium-derived factor with potent neuronal differentiative activity. *Exp Eye Res* 53(3): 411-414, 1991. PMID: 1936177. DOI: 10.1016/0014-4835(91)90248-d
- Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W and Bouck NP: Pigment epithelium-derived factor: A potent inhibitor of angiogenesis. *Science* 285(5425): 245-248, 1999. PMID: 10398599. DOI: 10.1126/science.285.5425.245
- Yoshida T, Akiba J, Matsui T, Nakamura K, Hisamoto T, Abe M, Ikezono Y, Wada F, Iwamoto H, Nakamura T, Koga H, Yamagishi SI and Torimura T: Pigment epithelium-derived factor (PEDF) prevents hepatic fat storage, inflammation, and fibrosis in dietary steatohepatitis of mice. *Dig Dis Sci* 62(6): 1527-1536, 2017. PMID: 28365916. DOI: 10.1007/s10620-017-4550-x
- Hou J, Ge C, Cui M, Liu T, Liu X, Tian H, Zhao F, Chen T, Cui Y, Yao M, Li J and Li H: Pigment epithelium-derived factor promotes tumor metastasis through an interaction with laminin receptor in hepatocellular carcinomas. *Cell Death Dis* 8(8): e2969, 2017. PMID: 28771223. DOI: 10.1038/cddis.2017.359
- Kawaguchi T, Yamagishi S, Itou M, Okuda K, Sumie S, Kuromatsu R, Sakata M, Abe M, Taniguchi E, Koga H, Harada M, Ueno T and Sata M: Pigment epithelium-derived factor inhibits lysosomal degradation of bcl-xl and apoptosis in Hepg2 cells. *Am J Pathol* 176(1): 168-176, 2010. PMID: 19948828. DOI: 10.2353/ajpath.2010.090242
- Uehara H, Miyamoto M, Kato K, Ebihara Y, Kaneko H, Hashimoto H, Murakami Y, Hase R, Takahashi R, Mega S, Shichinohe T, Kawarada Y, Itoh T, Okushiba S, Kondo S and Katoh H: Expression of pigment epithelium-derived factor decreases liver metastasis and correlates with favorable prognosis for patients with ductal pancreatic adenocarcinoma. *Cancer Res* 64(10): 3533-3537, 2004. PMID: 15150108. DOI: 10.1158/0008-5472.Can-03-3725
- Garcia M, Fernandez-Garcia NI, Rivas V, Carretero M, Escamez MJ, Gonzalez-Martin A, Medrano EE, Volpert O, Jorcano JL, Jimenez B, Larcher F and Del Rio M: Inhibition of xenografted human melanoma growth and prevention of metastasis development by dual antiangiogenic/antitumor activities of pigment epithelium-derived factor. *Cancer Res* 64(16): 5632-5642, 2004. PMID: 15313901. DOI: 10.1158/0008-5472.Can-04-0230
- Cheung LW, Au SC, Cheung AN, Ngan HY, Tombran-Tink J, Auersperg N and Wong AS: Pigment epithelium-derived factor is estrogen sensitive and inhibits the growth of human ovarian cancer and ovarian surface epithelial cells. *Endocrinology* 147(9): 4179-4191, 2006. PMID: 16777976. DOI: 10.1210/en.2006-0168
- Filleul S, Nelius T, de Riese W and Kennedy RC: Characterization of pedf: A multi-functional serpin family protein. *J Cell Biochem* 106(5): 769-775, 2009. PMID: 19180572. DOI: 10.1002/jcb.22072
- Chung C, Doll JA, Gattu AK, Shugrue C, Cornwell M, Fitchew P and Crawford SE: Anti-angiogenic pigment epithelium-derived factor regulates hepatocyte triglyceride content through adipose triglyceride lipase (ATGL). *J Hepatol* 48(3): 471-478, 2008. PMID: 18191271. DOI: 10.1016/j.jhep.2007.10.012
- Borg ML, Andrews ZB, Duh EJ, Zechner R, Meikle PJ and Watt MJ: Pigment epithelium-derived factor regulates lipid metabolism *via* adipose triglyceride lipase. *Diabetes* 60(5): 1458-1466, 2011. PMID: 21464445. DOI: 10.2337/db10-0845
- Bernard A, Gao-Li J, Franco CA, Bouceba T, Huet A and Li Z: Laminin receptor involvement in the anti-angiogenic activity of pigment epithelium-derived factor. *J Biol Chem* 284(16): 10480-10490, 2009. PMID: 19224861. DOI: 10.1074/jbc.M809259200
- Zagani R, El-Assaad W, Gamache I and Teodoro JG: Inhibition of adipose triglyceride lipase (ATGL) by the putative tumor suppressor g0s2 or a small molecule inhibitor attenuates the growth of cancer cells. *Oncotarget* 6(29): 28282-28295, 2015. PMID: 26318046. DOI: 10.18632/oncotarget.5061
- Montuori N and Sobel ME: The 67-kda laminin receptor and tumor progression. *Curr Top Microbiol Immunol* 213 (Pt 1): 205-214, 1996. PMID: 8814988. DOI: 10.1007/978-3-642-61107-0_13
- Wang X, Xiu P, Wang F, Zhong J, Wei H, Xu Z, Liu F and Li J: P18 peptide, a functional fragment of pigment epithelial-derived factor, inhibits angiogenesis in hepatocellular carcinoma *via* modulating VEGF/VEGFR2 signalling pathway. *Oncol Rep* 38(2): 755-766, 2017. PMID: 28627623. DOI: 10.3892/or.2017.5719
- The general rules for the clinical and pathological study of primary liver cancer. Edition Tt (ed.). Liver Cancer Study Group of Japan, 2015.
- Gao Y, Yao A, Zhang W, Lu S, Yu Y, Deng L, Yin A, Xia Y, Sun B and Wang X: Human mesenchymal stem cells overexpressing pigment epithelium-derived factor inhibit hepatocellular carcinoma in nude mice. *Oncogene* 29(19): 2784-2794, 2010. PMID: 20190814. DOI: 10.1038/ncr.2010.38
- Li C, Huang Z, Zhu L, Yu X, Gao T, Feng J, Hong H, Yin H, Zhou T, Qi W, Yang Z, Liu C, Yang X and Gao G: The contrary intracellular and extracellular functions of PEDF in HCC development. *Cell Death Dis* 10(10): 742, 2019. PMID: 31582735. DOI: 10.1038/s41419-019-1976-4
- Li F, Song N, Tombran-Tink J and Niyibizi C: Pigment epithelium-derived factor enhances differentiation and mineral deposition of human mesenchymal stem cells. *Stem Cells* 31(12): 2714-2723, 2013. PMID: 23939834. DOI: 10.1002/stem.1505
- Gattu AK, Swenson ES, Iwakiri Y, Samuel VT, Troiano N, Berry R, Church CD, Rodeheffer MS, Carpenter TO and Chung C: Determination of mesenchymal stem cell fate by pigment epithelium-derived factor (PEDF) results in increased adiposity and reduced bone mineral content. *Faseb j* 27(11): 4384-4394, 2013. PMID: 23887690. DOI: 10.1096/fj.13-232900
- Protiva P, Gong J, Sreekumar B, Torres R, Zhang X, Belinsky GS, Cornwell M, Crawford SE, Iwakiri Y and Chung C: Pigment epithelium-derived factor (PEDF) inhibits wnt/ β -catenin signaling in the liver. *Cell Mol Gastroenterol Hepatol* 1(5): 535-549.e514, 2015. PMID: 26473164. DOI: 10.1016/j.jcmgh.2015.06.006
- Hoshida Y, Nijman SM, Kobayashi M, Chan JA, Brunet JP, Chiang DY, Villanueva A, Newell P, Ikeda K, Hashimoto M, Watanabe G, Gabriel S, Friedman SL, Kumada H, Llovet JM and Golub TR: Integrative transcriptome analysis reveals common molecular subclasses of human hepatocellular

- carcinoma. *Cancer Res* 69(18): 7385-7392, 2009. PMID: 19723656. DOI: 10.1158/0008-5472.Can-09-1089
- 25 Chung C, Doll JA, Stellmach VM, Gonzales J, Surapureddi S, Cornwell M, Reddy JK and Crawford SE: Pigment epithelium-derived factor is an angiogenesis and lipid regulator that activates peroxisome proliferator-activated receptor alpha. *Adv Exp Med Biol* 617: 591-597, 2008. PMID: 18497086. DOI: 10.1007/978-0-387-69080-3_61
- 26 Utsunomiya T, Shimada M, Kudo M, Ichida T, Matsui O, Izumi N, Matsuyama Y, Sakamoto M, Nakashima O, Ku Y, Takayama T, Kokudo N and Liver Cancer Study Group of J: A comparison of the surgical outcomes among patients with HBV-positive, HCV-positive, and non-b non-c hepatocellular carcinoma: A nationwide study of 11,950 patients. *Ann Surg* 261(3): 513-520, 2015. PMID: 25072437. DOI: 10.1097/SLA.0000000000000821
- 27 Biagini G and Ballardini G: Liver fibrosis and extracellular matrix. *J Hepatol* 8(1): 115-124, 1989. PMID: 2646366. DOI: 10.1016/0168-8278(89)90170-0
- 28 Arriazu E, Ruiz de Galarreta M, Cubero FJ, Varela-Rey M, Pérez de Obanos MP, Leung TM, Lopategi A, Benedicto A, Abraham-Enachescu I and Nieto N: Extracellular matrix and liver disease. *Antioxid Redox Signal* 21(7): 1078-1097, 2014. PMID: 24219114. DOI: 10.1089/ars.2013.5697
- 29 Castera L, Hartmann DJ, Chapel F, Guettier C, Mall F, Lons T, Richardet JP, Grimbert S, Morassi O, Beaugrand M and Trinchet JC: Serum laminin and type iv collagen are accurate markers of histologically severe alcoholic hepatitis in patients with cirrhosis. *J Hepatol* 32(3): 412-418, 2000. PMID: 10735610. DOI: 10.1016/s0168-8278(00)80391-8
- 30 Hughes RC and Stamatoglou SC: Adhesive interactions and the metabolic activity of hepatocytes. *J Cell Sci Suppl* 8: 273-291, 1987. PMID: 3332663. DOI: 10.1242/jcs.1987.supplement_8.15
- 31 Ripoll C, Bari K and Garcia-Tsao G: Serum albumin can identify patients with compensated cirrhosis with a good prognosis. *J Clin Gastroenterol* 49(7): 613-619, 2015. PMID: 25203362. DOI: 10.1097/mcg.0000000000000207
- 32 Wang J, Zhang Z, Yan X, Li M, Xia J, Liu Y, Chen Y, Jia B, Zhu L, Zhu C, Huang R and Wu C: Albumin-bilirubin (ALBI) as an accurate and simple prognostic score for chronic hepatitis b-related liver cirrhosis. *Dig Liver Dis* 51(8): 1172-1178, 2019. PMID: 30765220. DOI: 10.1016/j.dld.2019.01.011
- 33 Ho TC, Chen SL, Shih SC, Wu JY, Han WH, Cheng HC, Yang SL and Tsao YP: Pigment epithelium-derived factor is an intrinsic antifibrosis factor targeting hepatic stellate cells. *Am J Pathol* 177(4): 1798-1811, 2010. PMID: 20709803. DOI: 10.2353/ajpath.2010.091085
- 34 Mejias M, Coch L, Berzigotti A, Garcia-Pras E, Gallego J, Bosch J and Fernandez M: Antiangiogenic and antifibrogenic activity of pigment epithelium-derived factor (PEDF) in bile duct-ligated portal hypertensive rats. *Gut* 64(4): 657-666, 2015. PMID: 24848263. DOI: 10.1136/gutjnl-2014-307138

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