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ORIGINAL ARTICLE

Basic Study

Phosphatase and tensin homolog is a differential diagnostic marker between nonalcoholic and alcoholic fatty liver disease

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Abstract

AIM: To investigate the protein expression of phosphatase and tensin homolog (PTEN) in human liver biopsies of patients with alcoholic and non-alcoholic liver disease.

METHODS: PTEN protein expression was assessed

by immunohistochemistry in formalin-fixed, paraffin-embedded liver sections of patients with non-alcoholic fatty liver disease (NAFLD) (n = 44) or alcoholic liver disease (ALD) (n = 25). Liver resections obtained from 3 healthy subjects candidate for partial liver donation served as controls. Histological evaluations were performed by two experienced pathologists, and diagnoses established based on international criteria. The intensity of the PTEN staining in nuclei was compared between steatotic and non-steatotic areas of each liver fragment analyzed. For each liver specimen, the antibody-stained sections were examined and scored blindly by three independent observers, who were unaware of the patients' clinical history.

RESULTS: In healthy individuals, PTEN immunostaining was intense in both the cytoplasm and nuclei of all hepatocytes. However, PTEN was strongly down-regulated in both the nucleus and the cytoplasm of hepatocytes from steatotic areas in patients with NAFLD, independently of the disease stage. In contrast, no changes in PTEN protein expression were observed in patients with ALD, regardless of the presence of steatosis or the stage of the disease. The degree of PTEN downregulation in hepatocytes of patients with NAFLD correlated with the percentage of steatosis (r = 0.3061, P = 0.0459) and the BMI (r = 0.4268, P = 0.0043). Hovewer, in patients with ALD, PTEN expression was not correlated with the percentage of steatosis with or without obesity as a confounding factor (P = 0.5574). Finally, PTEN expression level in steatotic areas of ALD patients was significantly different from that seen in steatotic areas of NAFLD patients (P < 0.0001).

CONCLUSION: PTEN protein expression is downregulated early in NAFLD, but not in ALD. PTEN immunohistochemical detection could help in the differential diagnosis of NAFLD and ALD.

Key words: Fibrosis; Phosphatase and tensin homolog; Steatosis; Non-alcoholic fatty liver disease; Nonalcoholic steatohepatitis; Alcoholic liver disease; Cirrhosis; Hepatocellular carcinoma

Core tip: Non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) display similar histopathological features making difficult to discriminate between them apart from patient history. In this report, we assessed the phosphatase and tensin homolog (PTEN) expression level by immunohistochemistry and observed that while PTEN was downregulated in steatotic hepatocytes from patients with NAFLD, its expression remained unchanged in patients with ALD. We therefore propose that the evaluation of PTEN expression could be a useful tool for the differential diagnosis of NAFLD and ALD.

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INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) are two major causes of chronic liver disease worldwide[1]. Both conditions display very similar histopathological features ranging from excessive accumulation of fat in the liver (steatosis) to steatohepatitis, fibrosis and cirrhosis[2]. Development of hepatocellular carcinoma (HCC) is a severe complication of both liver diseases. Liver biopsy is the gold standard method to confirm the diagnosis and to evaluate the degree of necroinflammatory activity and the stage of fibrosis. For the NAFLD, a scoring system (NAFLD activity score or NAS) may be useful to identify patients who are at risk to develop cirrhosis[3]. However, the differential diagnosis between ALD and NAFLD cannot be made on the basis of histological criteria alone, as it relies on patient's reported alcohol consumption, which is subject to the well-known self-reporting biases.

The phosphatase and tensin homolog deleted on chromosome 10 (PTEN) is a major tumor suppressor frequently mutated/deleted or downregulated in human cancers including HCC[4,5]. The tumor suppressive activity of PTEN appears to rely on multiple functions of this protein, including antagonism of PI3K signaling in response to growth factors[6], modulation of the activity of critical factors regulating cell adhesion, migration and invasion[7-9], and maintenance of chromosome stability and DNA repair[10]. In addition, the expression or activity of a whole network of other tumour suppressors frequently altered in human cancers can be modulated by dysregulated PTEN expression or activity[11]. Importantly, recent evidence indicated that in contrast to the classical "two hits" Knudson's hypothesis for tumour suppressors, PTEN haploinsufficiency, or even a partial loss of its activity/expression through post-transcriptional alterations, is sufficient to promote carcinogenesis in specific organs[12].

Conditional knockout of PTEN specifically in hepatocytes was shown to induce the sequential development of steatosis, fibrosis and HCC with ageing in mice[13]. Conversely, we previously demonstrated that PTEN expression/activity was downregulated in the liver of obese human and rodent models developing hepatic steatosis[14]. Further analyses revealed that PTEN expression was downregulated in cultured hepatocytes exposed to free fatty acids[15]. Together, these data indicated that alterations of PTEN expression/activity

represent an early event in NAFLD likely contributing to steatosis development. Surprisingly, studies in rodents, or using *in vitro* cell cultures, indicated that hepatic PTEN expression might be not decreased, but rather increased, with abusive alcohol consumption although histopathological alterations of the liver are very similar to those occurring in NAFLD[16-19]. However, whether these findings are relevant for human diseases is still unclear and the PTEN expression and activity status in the liver of patients with ALD is unknown.

Based on the above observations, we hypothesized that PTEN expression level in the liver may allow discriminating between NAFLD and ALD. Thus, we investigated the expression of PTEN by immunohistochemistry in liver biopsy samples from patients displaying all histopathological stages of NAFLD and ALD. Our results demonstrate that assessing PTEN protein expression in human livers may represent an important tool for the differential diagnosis of NAFLD and ALD.

MATERIALS AND METHODS

Human samples

Liver biopsy samples were collected between 2006 and 2015 from patients consecutively admitted at the Division of Gastroenterology and Hepatology of Geneva University Hospitals for investigation of abnormal liver function tests and fulfilling the diagnostic criteria of NAFLD or ALD[20]. Overall, a total of 69 patients were studied, including 44 obese patients with NAFLD, 17 non-obese patients with ALD and 8 obese patients with ALD. All patients had a negative serology for hepatitis B and C, tested negative for autoantibodies, have normal α-antitrypsin and ceruloplasmin serum levels and normal transferrin saturation. No patients were under medications that may induce steatohepatitis-like lesions. Patients with NAFLD were obese (Median BMI 41) and consume less than 10 gr of alcohol per day. Patients with ALD had a median BMI of 26 and declared daily alcohol intake above or equal to 40 gr for women and 60 gr for men at the time of liver biopsy. Liver resections obtained from 3 healthy subjects candidate for partial liver donation served as controls. Demographic data, including age, sex, body mass index (BMI) and alcohol consumption, were collected at the time of liver biopsy.

Histology and immunohistochemistry

Liver biopsies were formalin-fixed, paraffin-embedded and processed for histological staining. Histological evaluations were performed by two experienced pathologists, and diagnoses established based on international criteria[20]. NAFLD activity and fibrosis were graded using the NAS score[21]. Steatosis severity was scored as follows: 0 (none) $\leqslant$ 2%, 1 (mild) = 2%-30%, 2 (moderate) = 30%-60% and 3 (severe) $\geqslant$ 60% of hepatocytes affected.

All biopsy samples were processed together for the HE staining and PTEN immunohistological staining. Immunohistochemical analysis of PTEN expression was performed as previously described[14].

The intensity of the PTEN staining in nuclei was compared between steatotic (S) and non-steatotic (NS) areas of each liver fragment analyzed. Both S and NS areas from all slides were scored on a staining intensity scale from 0 to 4 with score = 0 for lack of staining, score = 1 for weak staining, score = 2 for moderate staining, score = 3 for strong staining and score = 4 representing the highest intensity. Then, the difference between NS and S areas (NS-S) was calculated. For each liver specimen, the antibody-stained sections were examined and scored blindly by three independent observers, who were unaware of the patients' clinical history.

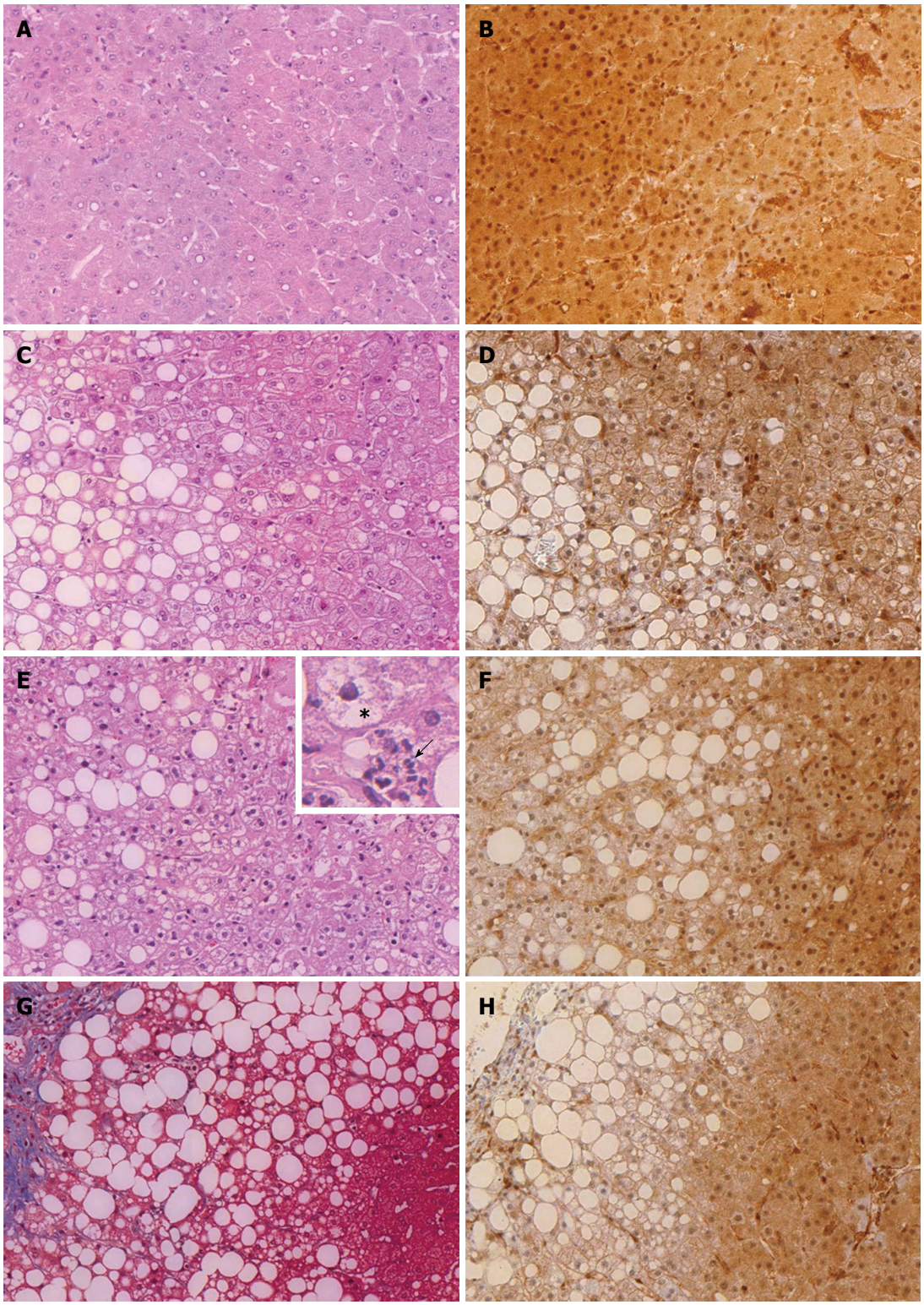
Statistical analyses

Statistical analyses were performed using one-way analysis of variance followed by Turkey test for continuous variables and nonparametric Spearman correlation for categorical variables.

RESULTS

PTEN expression is downregulated in steatotic hepatocytes from patients with NAFLD

PTEN expression was investigated in 44 patients with NAFLD at different stages of disease (steatosis n = 10, steatohepatitis without fibrosis n = 14, steatohepatitis with fibrosis n = 12 and cirrhosis n = 8) and compared to liver biopsy specimens from 3 healthy donors. In healthy individuals (Figure 1A and B), PTEN immunostaining was intense in both the cytoplasm and nuclei of all hepatocytes (Figure 1A and B). In the liver biopsies of patients with NAFLD, a significant decrease of PTEN expression was observed in steatotic hepatocytes, or in those in their close vicinity. Importantly, non-steatotic areas within the same specimen displayed similar immunostaining intensity than those of healthy controls, suggesting that PTEN expression was altered only in steatotic areas of NAFLD patients. Decrease of PTEN expression occurred in both the nucleus and the cytoplasm of hepatocytes. Nevertheless, only the nuclear staining was used to establish the PTEN score in order to prevent misevaluation of cytoplasmic PTEN staining potentially corrupted by the presence of large and unstained cytoplasmic lipid droplets in steatotic areas. When patients were divided according to the NAFLD stage, the expression of PTEN in steatotic areas was decreased in 9/10 patients with steatosis only (Figure 1C and D, Table 1), in 12/14 patients with steatosis and inflammatory infiltrate (Figure 1E and F, Table 1), in 11/12 patients with fibrosis (Figure 1G and H, Table 1) and 6/8 patients with cirrhosis (Figure 1I and J, Table 1). The degree of PTEN downregulation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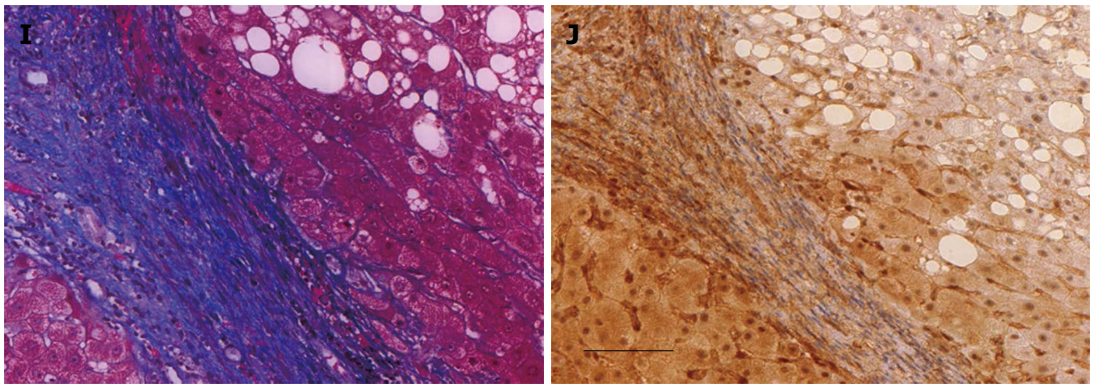

Figure 1 General histology and immunohistochemical detection of phosphatase and tensin homolog protein expression in the liver of healthy donor (hepatic resections) or patients with different stages of non-alcoholic fatty liver disease (liver biopsies). Liver sections of healthy donors (A and B) or of obese patients with steatosis (n = 10, C and D), nonalcoholic steatohepatitis (n = 14, E and F), fibrosis (n = 12, G and H) and cirrhosis (n = 8, I and J) were either stained with hematoxylin eosin (A, C, E), Masson's trichrome (G and I) or immunostained with anti-PTEN antibody (B, D, F, H and J). The inset in image (E) shows hepatic intralobular inflammation characterized by neutrophils (arrow) and ballooning hepatocyte (star). Scale bar = 100 μm. PTEN: Phosphatase and tensin homolog.

Table 1 Demographic data, histological diagnosis of liver biopsies and phosphatase and tensin homolog expression in patients with non-alcoholic fatty liver disease

Case	Age/sex	BMI (kg/m^2)	Lobular inflammation	Hepatocyte ballooning	Steatosis score	Steatosis	Fibrosis	NAS score	Scores for PTEN expression		
									NS	S	NS-S
Controls											
1	86/M	ND	0	0	0	0%	0	0	4	/	/
2	32/F	ND	0	0	0	0%	0	0	4	/	/
3	36/M	ND	0	0	0	0%	0	0	3	/	/
Pure steatosis											
4	26/M	39	0	0	1	10%	0	1	4	2	2
5	46/M	43	0	0	1	30%	0	1	3	2	1
6	38/F	55	0	0	1	20%	0	1	4	2	2
7	36/F	34	0	0	1	10%	0	1	4	4	0
8	37/F	35	0	0	2	60%	0	2	3	1	2
9	45/M	54	0	0	2	50%	0	2	4	1	3
10	29/M	41	0	0	2	40%	0	2	4	1	3
11	60/F	39	0	0	3	80%	0	3	4	2	2
12	43/M	40	0	0	3	70%	0	3	4	1	3
13	45/M	42	0	0	3	70%	0	3	4	1	3
Steatohepatitis without fibrosis											
14	49/F	36	0	1	1	30%	0	2	2	2	0
15	48/F	58	0	1	2	50%	0	3	4	1	3
16	57/M	34	1	1	2	60%	0	4	3	3	0
17	56/F	47	0	1	3	70%	0	4	4	2	2
18	40/M	40	0	1	3	70%	0	4	3	2	1
19	38/F	45	1	2	3	80%	0	6	3	1	2
20	59/F	41	1	1	3	70%	0	5	4	1	3
21	50/M	50	1	1	3	70%	0	5	4	1	3
22	35/F	51	1	1	3	80%	0	5	3	2	1
23	54/F	44	1	1	2	50%	0	4	3	2	1
24	42/F	39	1	1	3	80%	0	5	4	1	3
25	51/F	43	1	2	2	50%	0	5	4	2	2
26	56/M	39	1	1	3	80%	0	5	3	2	1
27	39/F	40	1	1	3	70%	0	5	3	2	1
Steatohepatitis with fibrosis											
28	62/M	39	0	0	3	80%	1a	3	4	1	3
29	47/F	47	0	0	2	50%	1a	2	4	1	3
30	21/M	40	0	0	3	80%	1a	3	3	2	1
31	50/F	42	0	0	3	70%	1a	3	4	2	1
32	54/M	32	0	0	1	10%	1a	1	3	2	1
33	57/M	51	0	0	2	50%	1b	2	4	1	3
34	60/M	40	0	0	1	20%	1b	1	3	3	0
35	48/F	63	0	0	3	80%	1c	3	4	1	3
36	55/M	35	0	0	2	50%	2	2	4	1	3
37	40/F	45	0	0	3	70%	2	3	2	1	1
38	36/M	41	1	2	3	90%	3	6	4	1	3
39	59/M	36	1	2	2	60%	3	5	4	1	3

Cirrhosis											
40	61/M	ND	0	1	1	20%	4	2	3	3	0
41	51/F	38	1	0	1	20%	4	2	3	3	0
42	54/M	58	1	1	2	50%	4	4	4	1	3
43	64/F	35	3	2	1	20%	4	6	3	2	1
44	60/M	36	1	2	2	50%	4	5	2	1	1
45	68/F	48	2	1	2	50%	4	5	3	1	2
46	65/F	47	1	1	3	70%	4	5	4	1	3
47	54/F	42	2	1	3	70%	4	6	4	1	3

The intensity of the PTEN nuclear staining was scored in steatotic (S) and non-steatotic (NS) areas of biopsies. All slides from healthy (Controls) and obese patients were scored on a staining intensity scale from 0 to 4 with score = 4 representing the highest intensity. Difference between NS and S area (NS-S) was calculated. ND: Not determined; PTEN: Phosphatase and tensin homolog.

in hepatocytes of patients with NAFLD only weakly correlated with the percentage of steatosis (r = 0.3061, P = 0.0459) and the BMI (r = 0.4268, P = 0.0043), but not with the NAS score (P = 0.3061), sex (P = 0.3583), age (P = 0.7931), fibrosis stage (P = 0.7235), degree of lobular inflammation (P = 0.9822) or hepatocyte ballooning (P = 0.9728).

Steatosis in patients with ALD does not alter PTEN expression in hepatocytes

PTEN expression was then performed on paraffin-embedded liver specimens from 25 patients with ALD at different stages of disease (all had steatosis, 15 had inflammation, 13 had different degrees of fibrosis and 21 had cirrhosis) (Table 2). In contrast to NAFLD patients, the presence of steatosis in liver samples of patients with ALD was not associated with a significant decrease of PTEN expression. Indeed, the intensity of PTEN immunostaining was similar in steatotic and non-steatotic areas of each liver biopsy, unaffected by the stage of the disease or obesity, and always comparable to the PTEN staining of healthy liver controls (Figure 2). PTEN expression was therefore not correlated with the percentage of steatosis in patients with ALD with or without obesity as a confounding factor (P = 0.5574). Finally, the PTEN expression level in steatotic areas of ALD patients was significantly different from that seen in steatotic areas of NAFLD patients (P < 0.0001).

DISCUSSION

NAFLD and ALD are two frequent liver diseases that require each a specific clinical management[22]. Non-alcoholic steatohepatitis is primarily managed by lifestyle interventions, *i.e.,* body weight loss combined to increased physical activity[22-24] associated with selected insulin sensitizers[22,25] and vitamin E administration[22,26]. Patients with ALD require complete alcohol abstinence, and, in severe forms of steatohepatitis, a short course of steroids, which has been proven effective in reducing mortality[27]. Unfortunately, the differential diagnosis between NAFLD and ALD may be difficult, owing to the bias intrinsic to the self-reported nature of alcohol consumption. In addition, both NAFLD - in its steatohepatitis form - and ALD share similar histological features. However, macrovesicular steatosis is reportedly more frequent and severe in NAFLD than in ALD, whereas microvesicular steatosis, lobular inflammation, Mallory-Denk bodies, satellitosis, acute cholestasis, sclerosing hyaline necrosis and veno-occlusive disease occur more frequently in ALD than in non-alcoholic steatohepatitis[28]. Nevertheless, all these histological features remain non-specific. Although one study suggested a disease-specific pattern of fibrosis silver impregnation *i.e.,* lattice-like in non-alcoholic steatohepatitis and solid in ALD[29], a molecular marker easy to use and able to discriminate between NAFLD and ALD has not been identified yet. Here, we show the potential role of PTEN immunostaining as a differential diagnostic marker to discriminate between ALD and NAFLD. The PTEN expression was found to be decreased in steatotic areas of the liver of patients with NAFLD, while being preserved in patients with ALD, irrespectively of the presence of obesity, steatosis and/or the liver disease stage. However, it has to be acknowledged that our group of patients with ALD is small and somewhat heterogeneous with regards to fibrosis and inflammation. Nevertheless, if our results are confirmed at a largest scale, PTEN immunostaining could be a valuable routine diagnostic tool for differentiating ALD and NAFLD therefore helping the decision making process in the clinical management of these patients.

The mechanisms of lipid accumulation in NAFLD and ALD are only partially known: some appear to be shared, while others seem to follow disease-specific pathways. The unfolded protein response is activated in both NAFLD and ALD by oxidative stress, and leads to the increase of transcriptional factors, such as the sterol regulatory element binding proteins, involved in *de novo* fatty acid synthesis[30,31]. Adiponectin, an adipokine produced by adipocytes that stimulates fatty acid oxidation, is downregulated in both NAFLD[32] and ALD[33]. On the other hand, some mechanisms contributing to the development of steatosis might be disease-specific. For example, an augmented lipolysis in the adipose tissue leading to increased circulating free fatty acids has been reported to occur mainly in NALFD but not in ALD[30,34-36], although this difference has been recently challenged in rodent models[37]. Dysregulated immune mechanisms may also play an important role in the early events and progression of

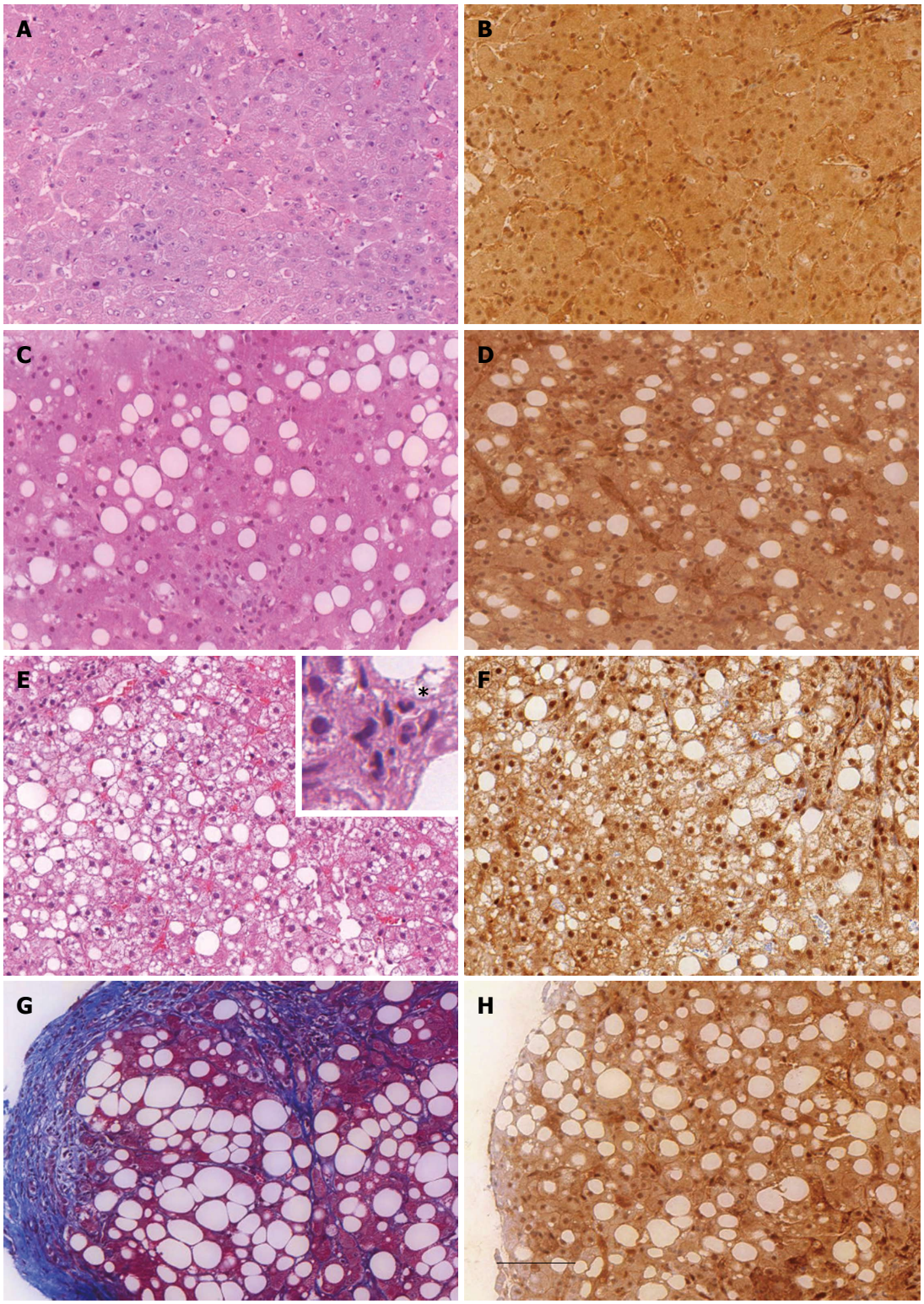

Figure 2 General histology and immunohistochemical detection of phosphatase and tensin homolog protein expression in the liver of healthy donor (hepatic resections) or patients with different stages of alcoholic liver disease (liver biopsies). Liver sections of healthy donors (A and B) or of patients with alcoholic steatosis (C and D), alcoholic steatohepatitis (E and F) and cirrhotic alcoholic disease (G and H) were stained with hematoxylin eosin (A, C and E), Massson's trichrome (G) or immunostained with anti-PTEN antibody (B, D, F and H). Scale bar = 100 μm. The inset in image (E) shows hepatic intralobular inflammation characterized by neutrophils (star). Scale bar = 100 μm. PTEN: Phosphatase and tensin homolog.

alcohol-related liver injuries[38].

Increasing evidence currently supports a key role for PTEN in the development of steatosis and fibrosis with distinct etiologies. First, mice bearing a hepatocyte-specific deletion of PTEN develop sequentially an extended hepatic steatosis, inflammation, fibrosis and HCC with ageing[13,39]. *In vitro* studies also showed that PTEN downregulation triggers lipid accumulation in

Table 2 Demographic data, histological diagnosis of liver biopsies and phosphatase and tensin homolog expression in patients with alcoholic liver disease

Case	Age/sex	BMI (kg/m^2)	Inflammation	Fibrosis	Cirrhosis	Steatosis	Score for PTEN expression		
							NS	S	NS-S
Non-obese patients									
1	56/F	26	-	p	-	100%	3	2	1
2	49/F	25	-	psn	-	40%	4	3	1
3	59/M	29	+	-	+	60%	3	3	0
4	57/F	27	+	-	+	80%	3	3	0
5	66/M	25	+	-	+	90%	4	3	1
6	55/F	28	+	-	+	90%	3	3	0
7	61/M	25	+	psn	+	70%	4	4	0
8	43/M	23	+	psn	+	80%	4	3	1
9	36/M	25	+	-	+	60%	3	2	1
10	41/M	24	+	psn	+	40%	4	3	1
11	64/M	21	+	-	+	10%	4	4	0
12	46/F	27	-	-	+	90%	3	3	0
13	28/F	22	+	-	+	80%	4	4	0
14	51/M	25	-	p	+	90%	4	3	1
15	43/M	20	+	psn	+	70%	3	3	0
16	54/M	20	-	psn	+	80%	4	4	0
17	36/M	23	-	psn	+	70%	3	2	1
Obese patients									
18	60/M	30	-	psn	-	70%	3	3	0
19	55/F	41	-	p	-	70%	3	3	0
20	57/M	33	+	-	+	80%	3	3	0
21	55/M	30	+	-	+	70%	3	2	1
22	51/M	32	-	-	+	20%	4	4	0
23	65/M	31	-	psn	+	5%	4	4	0
24	72/M	34	+	psn	+	90%	4	4	0
25	39/M	31	+	-	+	20%	4	4	0

The intensity of the PTEN staining was scored in steatotic (S) and non-steatotic (NS) areas of liver sections. All liver sections from ALD patients were scored on a staining intensity scale from 0 to 4 with score = 4 representing the highest intensity. Difference between NS and S area (NS-S) was calculated. ND: Not determined; Ctrl: Controls; psn: Perisinusoidal; p: Portal; PTEN: Phosphatase and tensin homolog; ALD: Alcoholic liver disease.

hepatoma cell lines[14,40]. Consistent with these studies, PTEN expression is decreased in steatotic livers of genetic and diet-induced obese rat models and in obese human subjects[14]. Hepatic PTEN downregulation was further reported in three different rodent models of liver inflammation/fibrosis induced either by a methionine/choline deficient diet[41], or bile-duct ligation[42] or CCL_4 administration[43]. In hepatitis C virus (HCV) infection, PTEN expression and/or activity were also reported to be altered in hepatocytes and to promote the development of steatosis[44]. PTEN downregulation was even reported to represent an independent prognostic factor for the survival of HCV-infected patients developing a cirrhotic HCC[45]. It thus appears that steatosis and its progression towards more severe hepatic disorders are associated with PTEN protein downregulation in the case of the metabolic syndrome and HCV infection.

The observation linking PTEN downregulation with liver disease progression in NAFLD and hepatitis C may have implications for treatment. Regular statin use has been shown to upregulate PTEN in heart tissue[46,47], skeletal muscles[48] and cancer cells[49-51] through various mechanisms including decreased expression of PTEN-targeting microRNAs[52], NF-κB inactivation[49] and PPARγ activation[50,51]. Regarding the relationship between statins and liver disease progression, the few data in NAFLD suggests some beneficial effect, at least on steatosis[53]. More convincing evidence has been reported in patients with chronic hepatitis C, where statin use was associated with a significantly lower liver fibrosis progression, independently of inflammation and viral load changes over time[54-56]. Thus, one cannot exclude that the beneficial effects of statins may be mediated by PTEN upregulation in the liver with NAFLD or HCV infection, however direct evidence are currently lacking. Obviously, the long-term use of statins in these conditions should also be weighed against the risk of toxicity, in particular concerning the increased risk of insulin resistance and type 2 diabetes development, which can results from PTEN upregulation in skeletal muscles[48,57,58].

Surprisingly, PTEN protein expression was reported increased in hepatoma cells exposed to ethanol[18] and in the liver of rats chronically fed with ethanol[16]. However, although PTEN expression is upregulated in the liver of ethanol-fed mice, the enzyme was shown to be highly carbonylated, a post-translational modification that decreases its phosphatase activity[59]. From these data, it results that steatosis in ALD is also associated in final with an impaired PTEN activity, although this defect is mediated by inhibition

of its enzymatic activity and not by repression of its expression as it is the case with obesity and HCV infection.

The mechanisms leading to PTEN protein up-regulation in hepatocytes exposed to alcohol are currently unknown. One attractive hypothesis is that PTEN transcription is stimulated by the transcription factor Egr1 in response to alcohol[60,61]. However, assessing whether PTEN expression is modulated at the mRNA level in human livers is technically challenging. Indeed, isolation of good quality mRNA from human liver biopsies processed for histological analysis is still poorly effective and cannot be used in routine diagnostic procedures. In addition, the high expression of a PTEN pseudogene (PTENP1) in human tissues can lead to misinterpretation of analyses aiming at assessing PTEN mRNA expression in pathological situations[62]. This is the reason why immunohistochemical detection of PTEN protein expression in clinical samples remains likely the gold standard method to assess PTEN expression in liver biopsies.

Our data and previous reports discussed here strongly support the concept that downregulation of either the expression, or the activity, of PTEN represents an important pathological mechanism contributing to the development of fatty liver diseases with distinct etiologies. However the mechanism affecting PTEN functions are different depending on the etiologies of the diseases. In this study, we show that we can take advantage of the different molecular mechanisms affecting PTEN activity or expression in liver metabolic disorders to identify their etiology. Thus, the immunohistochemical detection of PTEN protein expression should be added to the diagnostic armamentarium of pathologists and clinicians in the differential diagnosis of NAFLD and ALD in humans.

COMMENTS

Background

Similar histopathological alterations occur in patients suffering from non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD). Therefore, the differential diagnosis between ALD and NAFLD cannot be made on the basis of histological criteria alone, and it must rely on patient's reported alcohol consumption, which is subject to the well-known self-reporting biases. Studies in rodents, or *in vitro*, indicated that hepatic phosphatase and tensin homolog (PTEN) expression is decreased with obesity-associated steatosis, whereas it is increased with abusive alcohol consumption, although histopathological alterations of the liver are very similar. However, whether these findings are relevant for human diseases is still unclear and the PTEN expression and activity status in the liver of patients with ALD is unknown.

Research frontiers

Biomarker research in liver diseases is of growing importance not only to provide reliable differential diagnostic markers for therapeutic choices, but also as prognostic factors for progression of early stages of these diseases toward more severe pathologies such as cirrhosis and cancer.

Innovations and breakthroughs

Studies in rodents, or *in vitro*, indicate that alteration of PTEN function is a common event occurring at early stage of NAFLD, hepatitis C virus (HCV) infection and ALD. However, the molecular mechanisms underlining the loss of PTEN function are different, since they consist of the PTEN protein downregulation in NAFLD and HCV infection, whereas in ALD only PTEN activity is impaired but not its expression. In this study, the authors reported the differential expression of the PTEN protein in the liver of patients with NAFLD or ALD at various stages of these diseases, a finding of diagnostic significance.

Applications

Herein, the authors show that the different molecular mechanisms affecting PTEN expression in liver metabolic disorders can be an advantage to identify their etiology. Thus, the immunohistochemical detection of PTEN protein expression should be added to the diagnostic armamentarium of pathologists and clinicians in the differential diagnosis of NAFLD and ALD in humans.

Terminology

The "two hits" Knudson's hypothesis for tumour suppressors suggests that each allele of a tumour suppressor needs to be mutated, or deleted, to contribute to carcinogenesis, since one copy of a tumour suppressor is sufficient to ensure its function. Mallory-Denk bodies are damaged eosinophilic filamentous material forming inclusions in the cytoplasm of hepatocytes, in both ALD and NAFLD.

Peer-review

The article is very interesting and useful for clinicians. The small number of patients included in the study is a limit recognized by the authors, but most studies requiring liver biopsy in these types of pathology are difficult to achieve in a single center. A multicenter future study would be useful in this regard. Authors must underline the clinical implications of their research and add to discussion a comment on the beneficial role of statins, with regard to PTEN expression, in nonalcoholic fatty liver disease and in chronic hepatitis C.

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