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## Hepatocellular carcinoma and nonalcoholic steatohepatitis developing during long-term administration of valproic acid

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**Abstract** We report a case of hepatocellular carcinoma (HCC) arising in nonalcoholic steatohepatitis (NASH). The patient, a 64-year-old man, was incidentally found to have multiple tumors in the liver when admitted for pneumonia. He had been obese, had been receiving a standard dose of valproic acid since clipping surgery for subarachnoid hemorrhage 17 years previously, and had not consumed any alcohol since the surgery. Laboratory data revealed moderate hyperlipidemia and no evidence of diabetes mellitus, hepatitis B or C infection. The patient died of hepatic insufficiency, and an autopsy was performed. A tumor, a maximum of 13 cm in diameter, grossly occupied the entire left lobe and one third of the right lobe of the liver. Histologically, moderately differentiated HCC was found with foci of poorly differentiated HCC. The non-tumorous area showed NASH with moderate bridging fibrosis, without interface hepatitis, hemochromatosis, or copper accumulation. In this patient, obesity, hyperlipidemia, and long-term treatment with valproic acid could have all been associated with induction of NASH. The present case suggests that HCC could develop in non-cirrhotic NASH liver, and that chronic inflammation in itself could be an important risk factor in the development of HCC.

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### Introduction

Nonalcoholic steatohepatitis (NASH) is part of the spectrum of nonalcoholic fatty liver disease (NAFLD), clinically related with metabolic syndrome [3], and is histologically indistinguishable from alcoholic hepatitis [1]. Although the natural history of NASH has yet to be fully clarified, simple hepatic steatosis is nonprogressive, whereas nearly 20% of NASH cases may slowly progress to cirrhosis [8], and distinctive cases with hepatocellular carcinoma (HCC) arising in NASH have recently been reported [6, 13, 16, 17, 19, 23]. Valproic acid (VPA) is a worldwide antiepileptic drug, which occasionally causes idiosyncratic hepatotoxicity, but the association of VPA with steatohepatitis and HCC has not been determined [1]. This is the first report of HCC arising in moderately fibrosing NASH liver in a patient receiving long-term VPA administration.

### Clinical history

A 64-year-old man was admitted to hospital with a diagnosis of pneumonia. CT scans of the lower chest incidentally detected multiple tumors in the liver. The patient had received VPA (800 mg per day, standard dose) since clipping surgery for subarachnoid hemorrhage 17 years previously. He had been a chance drinker but had consumed no alcohol since the surgery 17 years previously. His family history was unremarkable. Laboratory data had irregularly shown slightly elevated liver enzymes. At the time of admission, total cholesterol (261 mg/dl) and triglyceride (175 mg/dl) levels were moderately increased, and the serum levels of  $\alpha$ -fetoprotein (18,270 ng/ml) and PIVKA-II (487 AU/ml) were highly elevated. The HbA<sub>1c</sub> value was within the normal range. Hepatitis B surface



**Fig. 1** Macroscopic appearance of the cut surface of the autopsied liver. The main tumor occupies the entire left lobe and approximately one third of the right lobe of the liver. There are also multiple nodules in the right lobe

antigen and anti-hepatitis C virus antibody in the serum were negative. Neither liver biopsy nor chemotherapy was performed. The patient died of hepatic insufficiency 1 year after admission, and an autopsy was performed 2 h after death.

## Materials and methods

The autopsy specimens were fixed in 10% formalin and embedded in paraffin. Tissue sections of 4  $\mu\text{m}$  in thickness were stained for hematoxylin and eosin as well as the Azan-Mallory method.

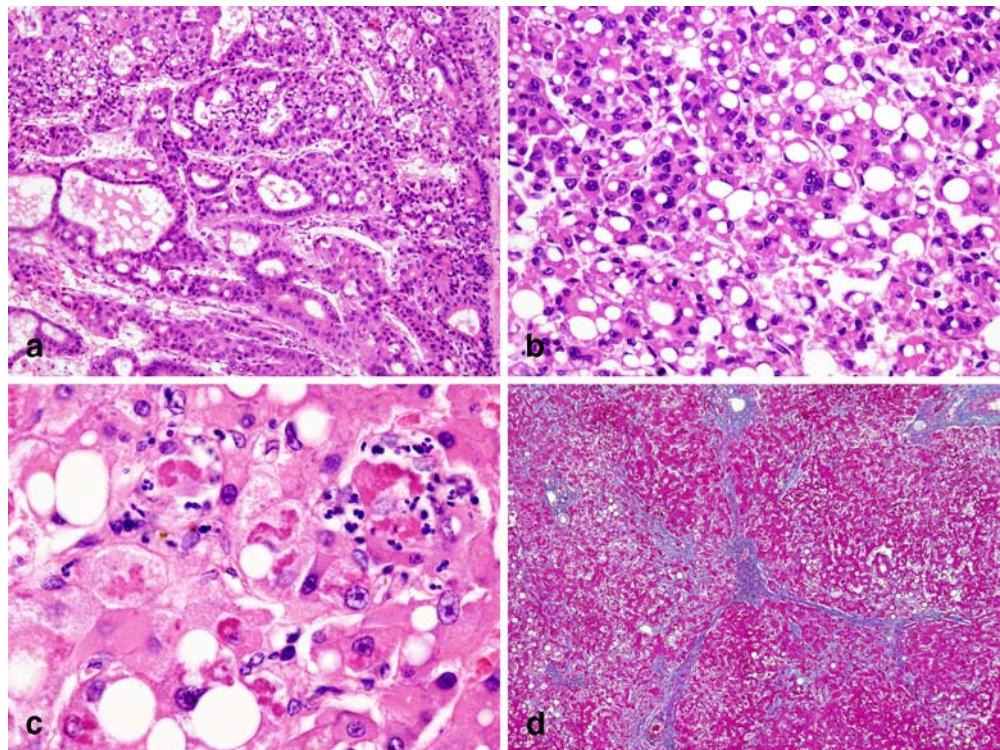
**Fig. 2** Histologic features of the tumor. **a** The tumor consists mainly of moderately differentiated hepatocellular carcinoma with a focal pseudoglandular pattern. Hematoxylin and eosin,  $\times 100$ . **b** There are hepatocellular carcinoma cells with marked fatty depositions. Hematoxylin and eosin,  $\times 200$ . **c** Ballooned liver cells with Mallory bodies and infiltrates of neutrophilic cells are frequently observed around the central vein. Hematoxylin and eosin,  $\times 400$ . **d** Bridging, perivenular, and pericellular fibrosis are diffusely observed in the non-tumorous area, but regenerative nodules are not visible. Azan-Mallory,  $\times 40$

## Results

At autopsy; the patient was obese, 82 kg in weight (BMI 34.1  $\text{kg}/\text{m}^2$ ), and 155 cm in height. The skin showed an icteric discoloration. Massive ascitic fluid (6,000 ml) was observed. The liver weighed 1,500 g, and the gross appearance showed that the tumor measured a maximum of 13 cm in diameter. The tumor occupied the entire left lobe and one third of the right lobe of the liver (Fig. 1). Histologic examination revealed that the tumor consisted mainly of moderately differentiated HCC, with a focal pseudoglandular pattern (Fig. 2a). A poorly differentiated HCC component was also observed in some parts of the tumor. HCC cells with marked fatty deposition were also observed focally (Fig. 2b). In the background of the tumor, both micro- and macrovesicular steatosis was apparent, especially in the centrilobular area. Ballooned liver cells with Mallory bodies and infiltrates of neutrophilic cells were commonly observed around the central veins (Fig. 2c). Spotty necrosis was scattered throughout the lobules. Perivenular and pericellular fibroses were also manifested. Bridging fibrosis between the central veins and portal tracts was evident, but regenerative nodules had not formed (Fig. 2d). There was no evidence of interface hepatitis, hemochromatosis, or copper accumulation.

## Discussion

This case is interesting as HCC developed in a non-cirrhotic NASH liver, and the patient had received long-term VPA therapy. The liver, in the presence of NASH, may have the potential to progress to cirrhosis and liver



**Table 1** Clinicopathologic features of cases with hepatocellular carcinoma and nonalcoholic steatohepatitis

Case	Author [reference]	Age/Sex	Background disease	HCC histology	Tumor size (cm)	Liver histology
1	Powell et al. [17]	57/F	DM	ND	ND	Cirrhosis
2	Cotrim et al. [6]	62/M	DM, OB	ND	3	Cirrhosis
3	Zen et al. [23]	72/F	DM	Mod	1.3	Cirrhosis
				Wel	1.0	
				Dys	1.4	
4	Orikasa et al. [16]	67/F	DM	Clear-cell	2.6	Pre-cirrhosis
5	Shimada et al. [19]	66/F	DM	Mod	1.5	Cirrhosis
6	Shimada et al. [19]	69/F	ND	Wel	1.5×2	Cirrhosis
7	Shimada et al. [19]	69/F	DM, OB	ND	2.5	Cirrhosis
8	Shimada et al. [19]	72/M	OB	Wel	3	Cirrhosis
9	Shimada et al. [19]	63/M	HL, OB	Wel	2	Cirrhosis
10	Shimada et al. [19]	56/M	DM	Mod	1–6, M	Cirrhosis
11	Mori et al. [13]	76/M	DM, OB	Wel	1.9	Cirrhosis
12	Present case	64/M	OB, HL, Drug	Mod	13, M	Fibrosis

F female, M male, DM diabetes mellitus, OB obesity, HL hyperlipidemia, HCC hepatocellular carcinoma, ND not described, Mod moderately differentiated, Wel well differentiated, Dys dysplasia, M multiple

failure, thereby promoting the development of HCC [17]. Although a large number of HCCs have been reported to arise in cryptogenic cirrhosis in patients with risk factors for NAFLD [4], the accurate incidence of HCC in NASH remains undetermined [3]. Half of the patients with cryptogenic cirrhosis have pathological and clinical characteristics consistent with NAFLD and account for 13% of HCC cases [12]. Some cases of cryptogenic cirrhosis have histologic features of burned-out NASH [15]. Cases of HCC that developed in NASH are not uncommon; however, to our knowledge, there have been only 11 well-documented cases reported in the literature [6, 13, 16, 17, 19, 23].

Table 1 summarizes the clinicopathologic features of the cases of HCC developing in NASH, including the present case. The histology of the background liver was cirrhosis in ten cases, and pre-cirrhosis in only one case. The present case is unique in that the tumor developed in a non-cirrhotic liver. More than 80% of patients with HCC have underlying cirrhosis [18], and cirrhosis itself is recognized as a common factor promoting HCC. However, the remaining cases of HCC, approximately 20%, can develop in a non-cirrhotic liver. There is a report of an animal study in which a new inbred mouse strain, fatty liver Shionogi, spontaneously developed fatty liver and HCC in the non-cirrhotic liver [21]. Although it remains uncertain whether cirrhosis is required for the development of HCC, at least, this case indicates that HCC can arise in a non-cirrhotic NASH liver.

Reactive oxygen species (ROS), products due to oxidative stress, activate lipid peroxidation, and TGF- $\beta$  induction, can stimulate fibrosis [1]. On the other hand, the major products of lipid peroxidation, malondialdehyde, and 4-hydroxynonenal, act as DNA mutagens [11], which might be associated with the initiation of HCC [5]. Because inflammation is a principal source of ROS, chronic inflammation can be a risk factor for various carcinomas [7].

Recently, mice with somatic inactivation of the *nrf1* gene in the liver have been reported to spontaneously develop HCC in a background of non-cirrhotic liver, and oxidative injury had been present before tumor development [22]. These results suggest that ROS overproduced in NASH liver could trigger carcinogenesis as well as fibrosis, and that cirrhosis is not essential for the development of HCC.

Major risk factors for primary NASH are: obesity, type 2 (non-insulin-dependent) diabetes mellitus, and hyperlipidemia [1]. Hyperinsulinemia is an underlying factor in these conditions. NAFLD was found to be present in almost 90% of patients with obesity; approximately 25% of whom had NASH [14]. About 50% of patients treated with VPA, which is speculated to modulate insulin secretion and systemic metabolism, experienced weight gain [2]. The present patient was obese and presented moderate hyperlipidemia, without evidence of diabetes mellitus. Obesity might be influenced by VPA and be indirectly associated with the development of HCC through the induction of NASH.

Causes of secondary NASH include: drugs, nutritional factors, and metabolic or genetic disorders [1]. VPA, a short fatty acid derivative, can cause hepatic microvesicular steatosis, which is related to insulin resistance [1]. VPA also impairs mitochondrial  $\beta$ -oxidation, a frequent mechanism underlying drug-induced steatohepatitis [9]. Derangements of mitochondrial  $\beta$ -oxidation generate an overproduction of ROS, leading to enhanced lipid peroxidation and cytokine production similar to primary (obesity-related) NASH [1]. These pathogenetic factors may act as an enhancement of HCC development. Contrarily, VPA has shown anticancer activity mediating inhibition of histone deacetylases and an induction of carcinoma cell differentiation [10, 20]. However, the antiproliferative effect on carcinoma cells of VPA and the relationship between VPA treatment and HCC development are not evident.

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