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CASE REPORT

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Hepatic adenocarcinoma expressing inhibin in a young patient on oral contraceptives

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Abstract A case of primary hepatic carcinoma is reported, which occurred in a 24-year-old woman with a 10-year history of oral contraceptive use, and demonstrated unique morphologic and immunohistochemical features. The tumor was located in the left hepatic lobe, measured 14 cm at its widest, and showed histologic heterogeneity. The neoplastic cells were mostly arranged in trabecular and pseudoglandular growth patterns simulating hepatocellular carcinoma; however, in focal areas, small cystic, organoid and tubular patterns predominated. Immunohistochemical stains showed a phenotype consistent with biliary differentiation (positive staining for cytokeratin 7, cytokeratin 19, carcinoembryonic antigen and CA 19-9 antigen). The tumor cells were negative for markers that would be suggestive of hepatocytic or neuroendocrine differentiation. Interestingly, they were positive for inhibin, a protein that is known to be expressed in sex cord–stromal tumors of the ovary, trophoblastic neoplasms and adrenal cortical tumors, but not in hepatic tumors. However, no definite evidence of gonadal stromal, trophoblastic, or adrenocortical differentiation was identified on extensive immunohistochemical work-up. In conclusion, this unique case may represent a rare variant of cholangiocarcinoma expressing inhibin.

Keywords Adenocarcinoma · Cholangiocarcinoma · Inhibin · Liver

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Introduction

Adenocarcinomas of the liver (intrahepatic cholangiocarcinomas) represent approximately 10% of primary carcinomas of this organ, and are thought to be derived from biliary epithelial cells. These tumors are known to be etiologically associated with parasitosis of the bile ducts, hepatolithiasis, congenital anomalies of the biliary tree, primary sclerosing cholangitis and Thorotrast exposure [2, 4]. Oral contraceptive steroids have also been suggested as possible etiologic agents of both intrahepatic and extrahepatic cholangiocarcinoma [1, 8]. In western countries, the majority of cholangiocarcinomas occur in patients without obvious predisposing factors.

While the commonest histologic appearance of cholangiocarcinoma is that of a tubular adenocarcinoma with abundant fibrous stroma, other architectural patterns may also be observed, including trabecular and papillary structures [2, 4]. Immunohistochemical stains typically show positivity for "biliary-type" cytokeratins (cytokeratins 7 and 19), carcinoembryonic antigen, epithelial membrane antigen, and antigen CA 19-9. Expression of inhibin is not a feature of either cholangiocarcinoma or any other primary hepatic tumor. This protein is commonly detected in gonadal stromal tumors and, occasionally, in other ovarian, trophoblastic and adrenal cortical neoplasms [3, 5–7, 9].

We report a unique case of hepatic adenocarcinoma with unusual histologic features, which expressed inhibin and occurred in a young patient on oral contraceptives.

Clinical history

A 24-year-old white woman presented with a 15-day history of nausea, vomiting and abdominal discomfort. Physical examination was remarkable for a palpable mass in the mid-upper abdomen. There were no other findings on complete systemic examination. The patient's past medical history was unremarkable. She was a non-smoker and occasionally consumed very small amounts of alcohol. She had

been on oral contraceptives (combination of 0.02 mg ethinylestradiol and 0.15 mg desogestrel) for 10 years.

Computed tomography (CT) scan of the abdomen showed a well-circumscribed mass, located in the left hepatic lobe and occupying the inferior and lateral borders. On administration of intravenous contrast, the lesion showed inhomogeneous enhancement, which was more intense on late images (Fig. 1a). On magnetic resonance images, the mass appeared well defined and measured 16.7×16.2 cm. It was multilobulated and inhomogeneous, with cystic changes, and showed enhancement after intravenous administration of paramagnetic contrast material (Fig. 1b). Biochemistry and hematology results were within normal limits. The serum levels of tumor markers, including carcinoembryonic antigen, CA 19-9 antigen and CA 125 antigen, were also within normal limits. A full hormonal screen, including follicle stimulating hormone, luteinizing hormone, prolactin, estradiol, progesterone, testosterone, dehydroepiandrosterone-S and thyroid-stimulating hormone, did not reveal any abnormality.

At laparotomy, the tumor mass was found to involve hepatic segments II and III and appeared to have a "pushing" rather than infiltrating border. A left lobectomy was performed. The lesion was excised intact, together with a 3-cm rim of hepatic parenchyma. The patient had an uneventful postoperative course and was discharged home 7 days following surgery. Since then (almost 3 years ago), she has been on follow-up with physical examination and tumor markers every 3 months, abdominal ultrasound scan every 6 months, and CT scan of thorax and abdomen every year. She has remained free of recurrence. No further treatment has been given.

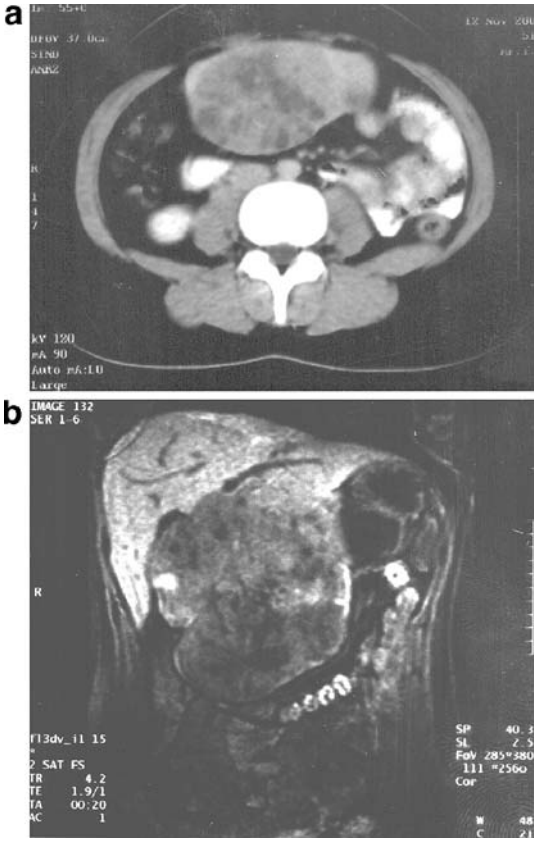

Fig. 1 Radiographic findings: **a** Abdominal CT scan showing a well-circumscribed tumor mass in the left hepatic lobe. **b** MRI of the abdomen: on fat saturation images, the tumor appears multilobulated and inhomogeneous, with focal cystic changes

Table 1 Summary of immunohistochemical stains. *HepPar-1* hepatocyte specific antigen; *CEA* carcinoembryonic antigen; *hCG* human chorionic gonadotropin; *NSE* neuron-specific enolase; *ER* estrogen receptor; *PR* progesterone receptor; *PAP* prostatic acid phosphatase

Antigen	Source of antibody	Clone	Result
Pancytokeratin	DAKO	AE1/AE3	Moderately positive
Cytokeratin 8/18	Becton Dickinson	cam5.2	Moderately positive
Cytokeratin 7	DAKO	OV TL 12/30	Strongly positive
Cytokeratin 19	DAKO	RCK108	Strongly positive
Cytokeratin 20	DAKO	Ks 20.8	Negative
HepPar-1	DAKO	OCH1E5	Negative
pCEA	DAKO	Polyclonal	Positive (cytoplasmic and membranous)
mCEA	DAKO	11-7	Negative
EMA	DAKO	E29	Negative
CA 19-9	DAKO	116-NS-19-9	Focally positive
CA 125	Novocastra	NCL-CA125	Negative
α-Fetoprotein	Signet	Polyclonal	Negative
hCG	Biocare	Polyclonal	Negative
NSE	Biocare	DT01-BC10	Negative
Chromogranin	Sanbio	Polyclonal	Negative
Synaptophysin	Ventana	Polyclonal	Negative
Somatostatin	Chemicon	Polyclonal	Negative
Insulin	DAKO	Polyclonal	Negative
Thyroglobulin	DAKO	DAK-Tg6	Negative
Calcitonin	Biogenex	Polyclonal	Negative
Vimentin	Biocare	V-9	Negative
Inhibin	DAKO	R1	Strongly positive
ER protein	DAKO	1D5	Negative
PR protein	Novocastra	1A6	Negative
PAP	DAKO	PASE/4LJ	Negative
S-100 protein	DAKO	Polyclonal	Rare positive cells
HMB-45	DAKO	HMB-45	Negative
CD10	Novocastra	56C6	Negative
CD34	Biogenex	Qbend/10	Negative
CD56	Novocastra	ERIC-1	Moderately positive
CD117 (c-kit)	Biotechnology	C-19	Negative
Ki-67	Immunotech	MIB1	5–10% of nuclei positive
p53	DAKO	D0-7	Negative

Materials and methods

Multiple sections of tumor and non-tumorous hepatic parenchyma were routinely processed for paraffin sections. Sections 4 μm thick were stained with hematoxylin–eosin and mucicarmine. Additional paraffin sections were used for immunohistochemical stains with a standard streptavidin–biotin staining protocol. The primary antibodies utilized and their sources are shown in Table 1.

Results

The surgical specimen weighted 940 g and measured 15× 14×7.5 cm. On sectioning, the specimen was almost com-

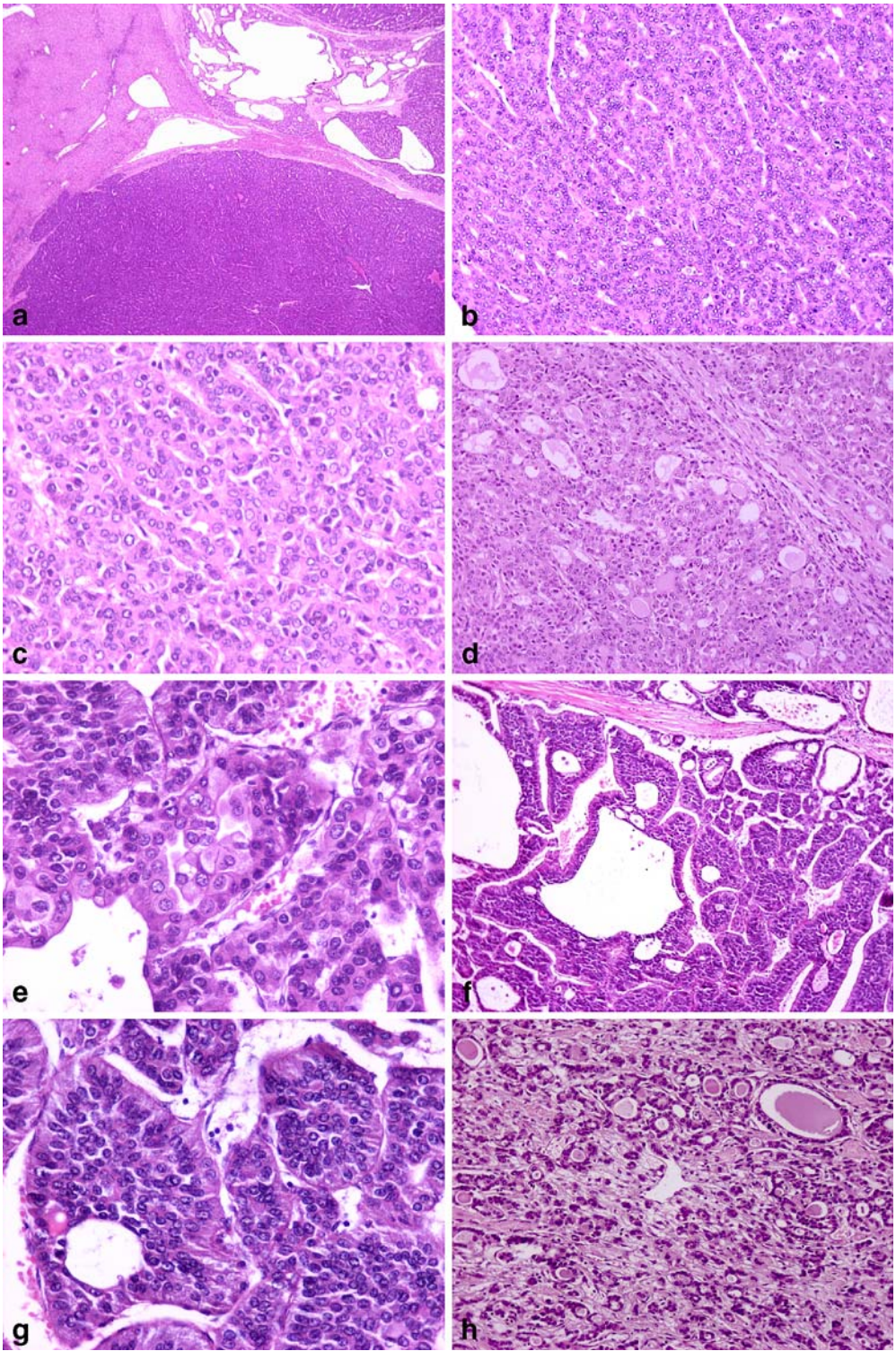

Fig. 2 Microscopic features. **a** Low-power view of the tumor shows areas of trabecular (bottom) and microcystic (top) architecture. **b** The trabeculae vary in thickness and are lined by endothelial cells. **c** High-power view shows cuboidal or columnar neoplastic cells with moderate nuclear pleomorphism. **d** In some areas, the cells form prominent pseudoglandular structures. **e** Occasional clusters of tumor cells with hepatocytoid features are present. **f**, **g** Focally, the tumor cells are arranged in organoid structures. **h** In a different area, the tumor cells form tubular structures within a loose fibrous stroma. (Original magnifications: **a**: ×20; **b**, **d**, **h**: ×100; **c**: ×200; **e**, **g**: ×400; **f**: ×40)

pletely occupied by a well-demarcated tumor, measuring 14 cm at its widest. The cut surface was tan, solid, with focal areas of microcystic change and extensive areas of hemorrhage.

Microscopic examination showed a carcinoma with predominantly trabecular architecture (Fig. 2a, b). The tumor cells were columnar or cuboidal, with roundish, hyperchromatic nuclei and moderate amounts of eosinophilic or amphophilic cytoplasm (Fig. 2c). Mitotic activity was mild. The trabeculae were several cells thick, and were lined by endothelial cells. The neoplastic cells within the trabeculae were often arranged in pseudoglandular structures, some-

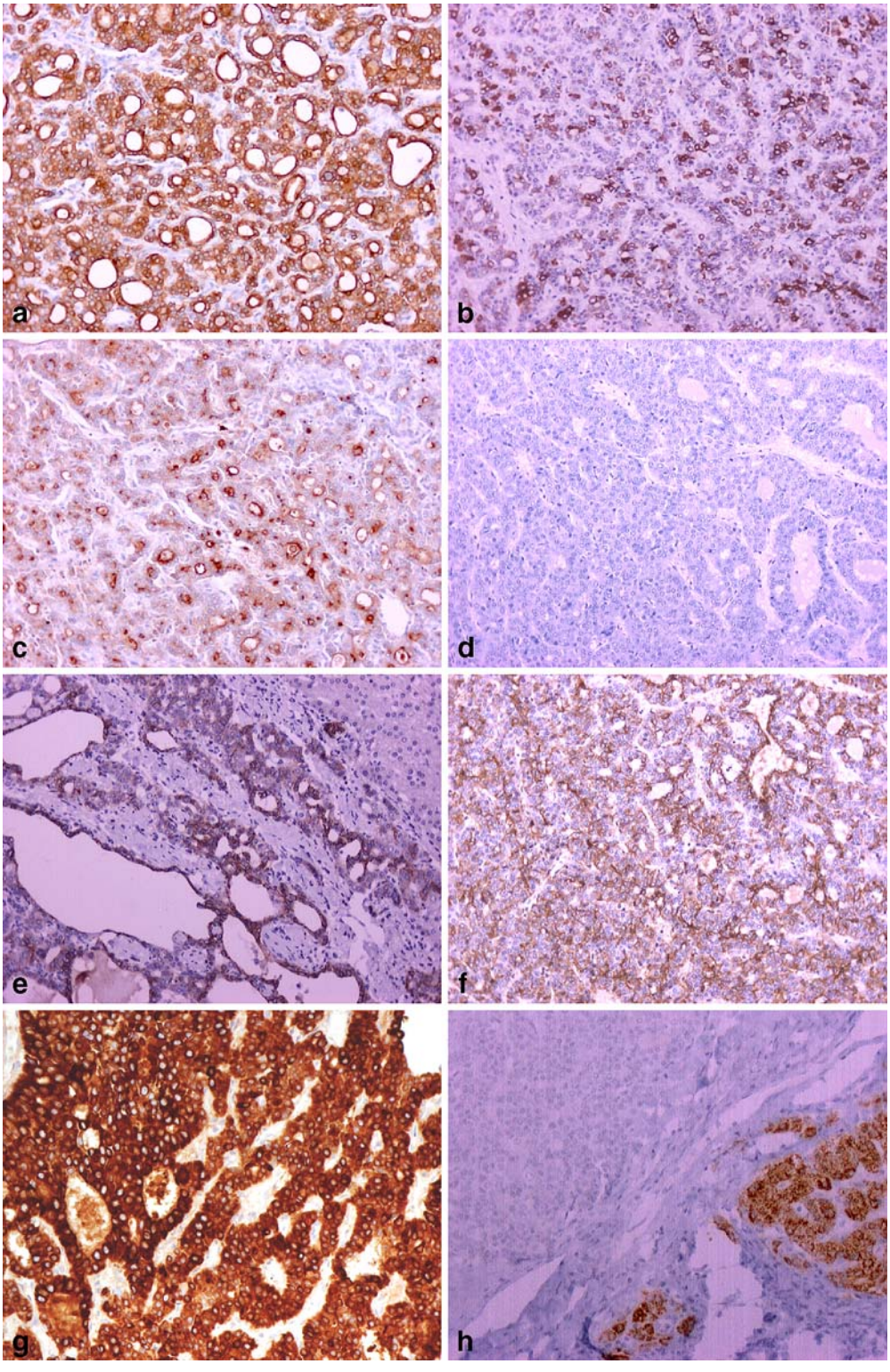

Fig. 3 Immunohistochemical findings: the tumor cells are positive for cytokeratin 7 (**a**) and cytokeratin 19 (**b**). Stains for carcinoembryonic antigen demonstrate cytoplasmic and membranous positivity with polyclonal (**c**), but not with monoclonal antibodies (**d**). The tumor cells are also positive for CA 19–9 (**e**), CD56 antigen (**f**) and inhibin (**g**). No positivity for HepPar-1 is seen in the neoplasm, as contrasted to nearby hepatocytes (**h**). (Original magnifications: **a**–**f**: ×100; **g**, **h**: ×200)

times containing amorphous eosinophilic material in the lumina (Fig. 2d). No bile production was seen. Mucicarmin stain was negative for mucin. In the central portion of some trabeculae, the tumor cells contained increased amounts of eosinophilic cytoplasm, resembling hepatocytes (Fig. 2e). In some areas, the neoplastic cells lined small cystic spaces (Fig. 2a, f) or formed organoid structures (Fig. 2f, g). In a small area close to the tumor edge, the tumor cells formed tubular structures, surrounded by a loose fibroconnective tissue stroma (Fig. 2h). The tumor contained foci of hemosiderin deposition, small foci of necrosis and occasional microcalcifications. The surrounding hepatic parenchyma appeared compressed, without evidence of pre-existing disease. The surgical margins of resection were free of tumor.

Due to the unusual histologic features of the tumor, extensive immunohistochemical stains were performed (Table 1). The neoplastic cells were positive for "biliary-type" cytokeratins (cytokeratins 7 and 19), carcinoembryonic antigen (cytoplasmic and membranous staining with polyclonal, but not with monoclonal antibodies), CA 19-9 antigen and CD56 antigen (Fig. 3a–f). They were also positive for inhibin (Fig. 3g). The tumor cells were negative for a variety of other markers, including those which would be suggestive of hepatocytic or endocrine differentiation (Table 1, Fig. 3h).

Discussion

Several aspects of the case reported herein are unusual. In the absence of chronic liver disease, hepatic carcinomas are rare in the 3rd decade of life, and, as a rule, represent hepatocellular carcinomas of the fibrolamellar variant. Our case did not demonstrate histologic features characteristic of fibrolamellar carcinoma. Since our patient did not have any other predisposing factor for tumor development, attention must be focused on the 10-year-long history of oral contraceptive use. In an analysis of possible etiologic factors in a series of 42 patients with intrahepatic cholangiocarcinoma, Altaee et al. found that 35% of the women had used oral contraceptives [1]. In a different study, a statistically significant association between the use of contraceptives and extrahepatic bile duct cancer was observed among women under 60 years of age [8].

The histologic features of our case are also unusual. In most areas, the predominant growth patterns were trabecular and pseudoglandular, simulating hepatocellular carcinoma. In several trabeculae, a neoplastic cell subpopulation with features suggestive of hepatocellular differentiation was also found. However, the tumor cell immunophenotype (cytokeratin 7 and 19 positive, carcinoembryonic antigen positive, CA 19-9 positive, hepatocyte specific antigen negative, α-fetoprotein negative) was consistent with a biliary tumor. Immunopositivity for CD56 antigen may also be supportive of biliary differentiation [10]. Because of the tendency of the neoplastic cells to form organoid structures, a variety of markers of endocrine differentiation were assessed, but were found to be negative.

The most unusual feature of this case was the strongly positive immunostaining for inhibin. This glycoprotein is known to be synthesized by granulosa cells, luteinized thecal cells, and hilus cells of the normal ovary, and to regulate reproductive functions in conjunction with activin [3]. Inhibin is widely accepted as a marker for sex cord tumors, especially for granulosa cell tumors, and appears to be helpful in the differential diagnosis of such tumors from other neoplasms affecting the ovary [5]. In addition, inhibin is expressed by syncytial trophoblastic cells and in neoplasms of trophoblastic origin, such as hydatidiform moles, placental site nodule, placental trophoblastic tumors, and choriocarcinoma [3, 7]. This protein is also expressed by the majority of adrenocortical adenomas and carcinomas [6, 9].

Inhibin expression is not considered a feature of primary hepatic neoplasms. On immunohistochemical studies, both hepatocellular carcinoma and cholangiocarcinoma have been found to be negative for this protein, with the exception of a single case of high-grade pleomorphic hepatocellular carcinoma [11–13]. In the absence of histologic and immunohistochemical evidence of gonadal stromal, trophoblastic or adrenocortical differentiation in the tumor we are reporting, we must conclude that this neoplasm represents a unique case of cholangiocarcinoma expressing inhibin. A possible association with oral contraceptive use may be suggested, but cannot be proven at the present time.

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