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Case Report

Unusual biliary myoepithelial carcinoma in liver-case report and immunohistochemical study

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Abstract: Myoepithelial carcinoma is a well-known tumor of salivary gland, representing 1% of all salivary gland tumors. They have also been reported in other sites as skin/soft tissue, breast and lung. This paper reports a rare case of primary myoepithelial carcinoma in the liver, as well as discusses the findings of immunohistochemistry. The clinical manifestations, imaging characteristics, and histopathological changes of myoepithelial carcinoma in this case were described. The patient was a 33 years old female presented with a cystic tumor in the right lobe of the liver. As the liver tumor increased in size within six months, malignant neoplasm was suspected and thus anterior hepatic segmentectomy was performed. The mass composed of glandular-like structures and trabecular sheets of spindled shaped cells and epithelioid cells which were positive for myoepithelial markers. The tumor recurred within one year, in the left lobe of the liver and partial left lobe lobectomy was performed. The tumor resected showed similar histology to the primary tumor. Three months later, another recurrence was noted for which radiofrequency ablation was performed. This report presents a recurrent case of myoepithelial carcinoma in the liver and suggests the possibility of biliary origin of such tumor.

Keywords: Recurrent myoepithelial carcinoma, liver, immunohistochemistry

Introduction

Myoepithelial carcinoma is a rare, locally aggressive salivary gland neoplasm, arising from myoepithelial cells that surround acini and ducts of salivary glands. The tumor shows a spectrum of cellular and architectural morphologies, as myoepithelial cells may be spindle shaped, plasmacytoid, ovoid, or epithelioid, and can express a variety of cytoplasmic filaments, such as cytokeratin and muscle filaments [1, 2]. Myoepithelial carcinomas are locally aggressive neoplasms which can range from intermediate grade to high-grade carcinoma [1]. Histologic grade does not appear to correlate well with clinical behavior, as tumors with a low-grade histologic appearance may behave aggressively. Approximately one third of patients die of disease, another third have recurrences, mostly multiple, and the remaining third are disease free [2]. These commonly occur in salivary glands, especially parotid gland [1, 3], but they have also been reported in the other sites [4-9]. However, to our knowledge there has been no report of this tumor in the liver. We report herein the first definite case of myoepithelial carcinoma arising in the liver, most probably from intrahepatic biliary ducts.

Case report

Clinical presentation

A 33-year-old-woman was referred for annual medical checkup. The patient had no subjective symptoms or significant past medical history. Physical examination revealed no abnormal signs. Blood test showed no abnormality; carcinoembryonic antigen (CEA) [1.5 ng/ml] and cancer antigen 19-9 [5.4 U/ml] were within normal limits, and hepatitis B surface antigen and hepatitis C virus antibody were negative. However,

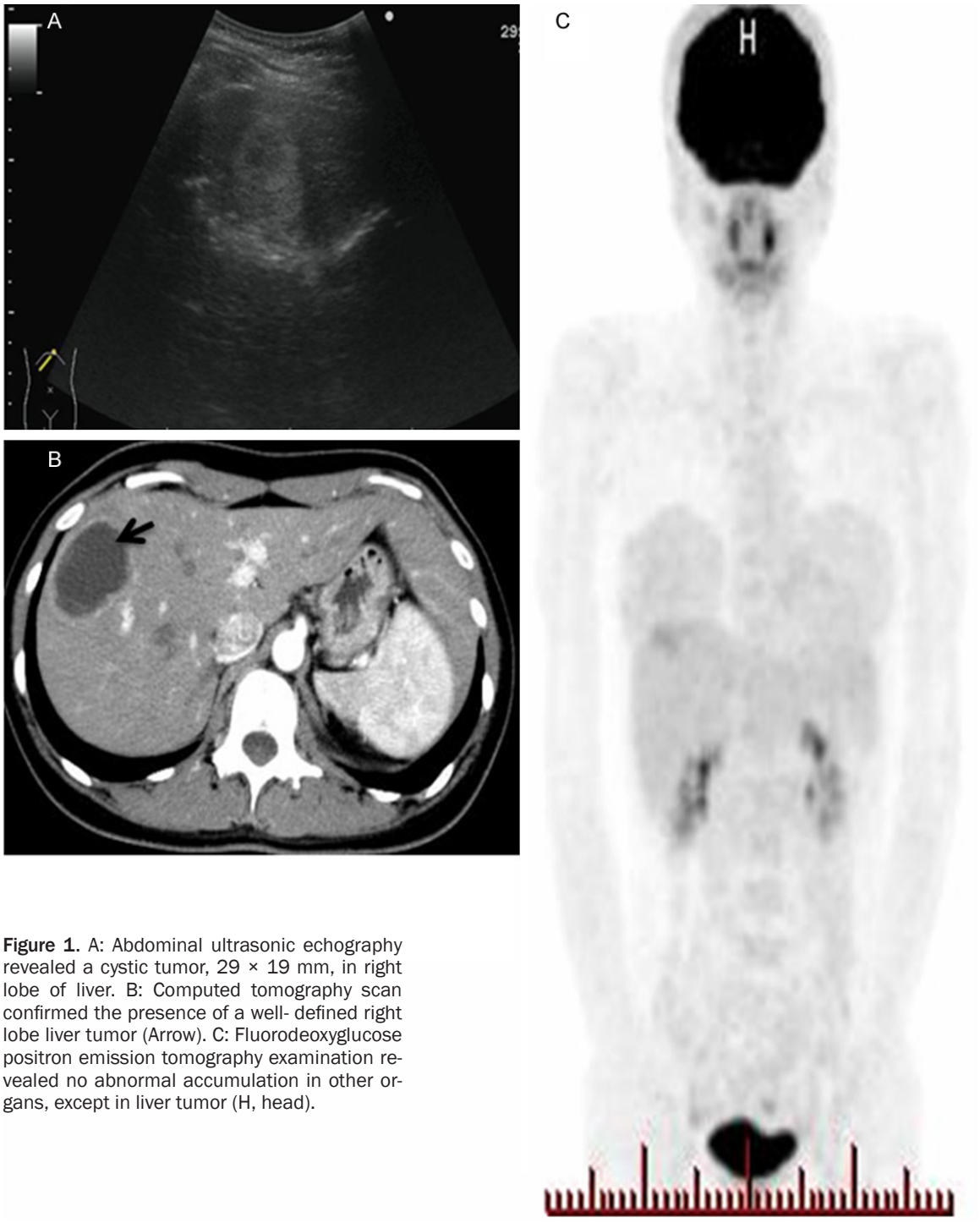

Figure 1. A: Abdominal ultrasonic echography revealed a cystic tumor, 29 × 19 mm, in right lobe of liver. B: Computed tomography scan confirmed the presence of a well- defined right lobe liver tumor (Arrow). C: Fluorodeoxyglucose positron emission tomography examination revealed no abnormal accumulation in other organs, except in liver tumor (H, head).

abdominal ultrasonic echography (**Figure 1A**) revealed a cystic tumor with a thick wall, 29 × 19 mm, in right lobe of liver. Hepatic hemangioma was suggested as an initial diagnosis. Computed tomography scan (**Figure 1B**) confirmed the ultrasonic echography findings. After 2 months later, magnetic resonance imaging examination revealed that the cystic tumor increased in size. Amoebic liver abscess, metastatic liver tumor and cystadenocarcinoma were considered, but upper and lower alimentary tract endoscopic examination and stool analysis showed no abnormalities. Follow up ultrasonic echography revealed

Table 1. Liver myoepithelial carcinoma, Immunohistochemical expression. References, lot and working dilutions of antibodies are indicated

Primary antibodies	Reference	Lot	Working dilution	Tumor cells
AFP	Sigma Aldrich, Ontario, Canada	C3	1:50	Negative
HepPar-1	DAKO, Glostrup, Denmark	OCH1E5	1:50	Negative
TTF-1	DAKO	8G7G3/1	1:100	Negative
Glypican 3	Gentaur, Kampenhout, Belgium	418021F	1:1	Negative
CK7	DAKO	OV-TL 12/30	1:50	Positive in entrapped bile ducts
CK 19	DAKO	M0888	1:100	Positive in entrapped bile ducts
CEA	DAKO	A0115	1:200	Positive
CAM 5.2	BD Bioscience, NJ, US		1:50	Focal positive
CK14	Leica, Wetzlar, Germany	NCL-LL002	1:100	Negative
AE1/AE3	DAKO		1:50	Positive, especially in epithelioid cells
EMA	DAKO	M0613	1:100	Positive, especially in squamoid cells
Desmin	DAKO	D33	1:100	Focal positive
GFAP	DAKO	Z0334	1:100	Positive
S-100	Amersham, Buckinghamshire, England		1:300	Positive
SMA	DAKO	M0851	1:50	Positive
Vimentin	DAKO	Mo725	1:400	Focal positive
CD10	Leica		1:25	Positive
p63	NeoMarkers, Fremont, CA	4A4	1:25	Occasionally weakly positive
Calponin	DAKO	M3556	1:20	Negative
Ki-67	DAKO	M7240	1:100	Positive in 10-20% of cells

AFP, alpha-feto protein; HepPar-1, Hepatocyte antigen in paraffin-1; TTF-1, thyroid transcription factor-1; CK, cytokeratin; CEA, carcinoembryonic antigen; EMA, epithelial membrane antigen; GFAP, glial fibrillary acidic protein; SMA, smooth muscle actin; PAS, periodic acid-Shiff; di-PAS, PAS after diastase; Ki-67, proliferation marker.

that the tumor size increased to 35 mm in greatest diameter within 3 months. FDG-PET examination revealed no abnormal accumulation in the other organs, except for the liver tumor (**Figure 1C**). The initial clinical diagnosis was malignant liver neoplasm and the patient underwent anterior hepatic segmentectomy.

Materials and methods

Surgical specimens were fixed in 10% neutral buffered formalin and embedded in paraffin. Serial sections, 5 μm thick, were processed for hematoxylin and eosin (H&E) staining and for mucin histochemistry with Alcian blue, periodic acid-Shiff (PAS) and PAS after diastase digestion (di-PAS). Immunohistochemical staining was carried out with the streptavidin-biotin method. List of primary antibodies used are listed in **Table 1**. This was followed by sequential 60 min incubations with secondary antibodies (Envision+System-HRP Labelled Polymer, DAKO) and visualization with the Liquid DAB+Substrate Chromogen System (DAKO). All slides are lightly counterstained with hematoxylin for 30s prior to dehydration and mounting.

Results

Pathological findings

The specimen consisted of the anterior hepatic segment, showing a single well-defined mass, located under the liver capsule, measuring 5 × 4 cm in size, with central cyst formation, due to necrosis (**Figure 2A**). Histologically, observed by H&E staining, the tumor was well delineated, partially encapsulated and formed from trabecular sheets and nests of cells with clear cytoplasm and areas of epithelioid cells with esinophilic cytoplasm (**Figure 2B**). The tumor cells invaded into the surrounding liver tissues (**Figure 2C**). With high power examination, the sheets and nests were formed of cells that are round to spindle shaped; some with clear cytoplasm and others with esinophilic cytoplasm (**Figure 2D**). Few scattered squamoid cells were seen (**Figure 2D**). Tumor cells had mild to moderate cellular and nuclear atypia, with slightly irregular nuclei and inconspicuous to small nucleoli (**Figure 2D**). There was no epithelial mucus production as demonstrated by Alcian blue staining. The clear cells demonstrated

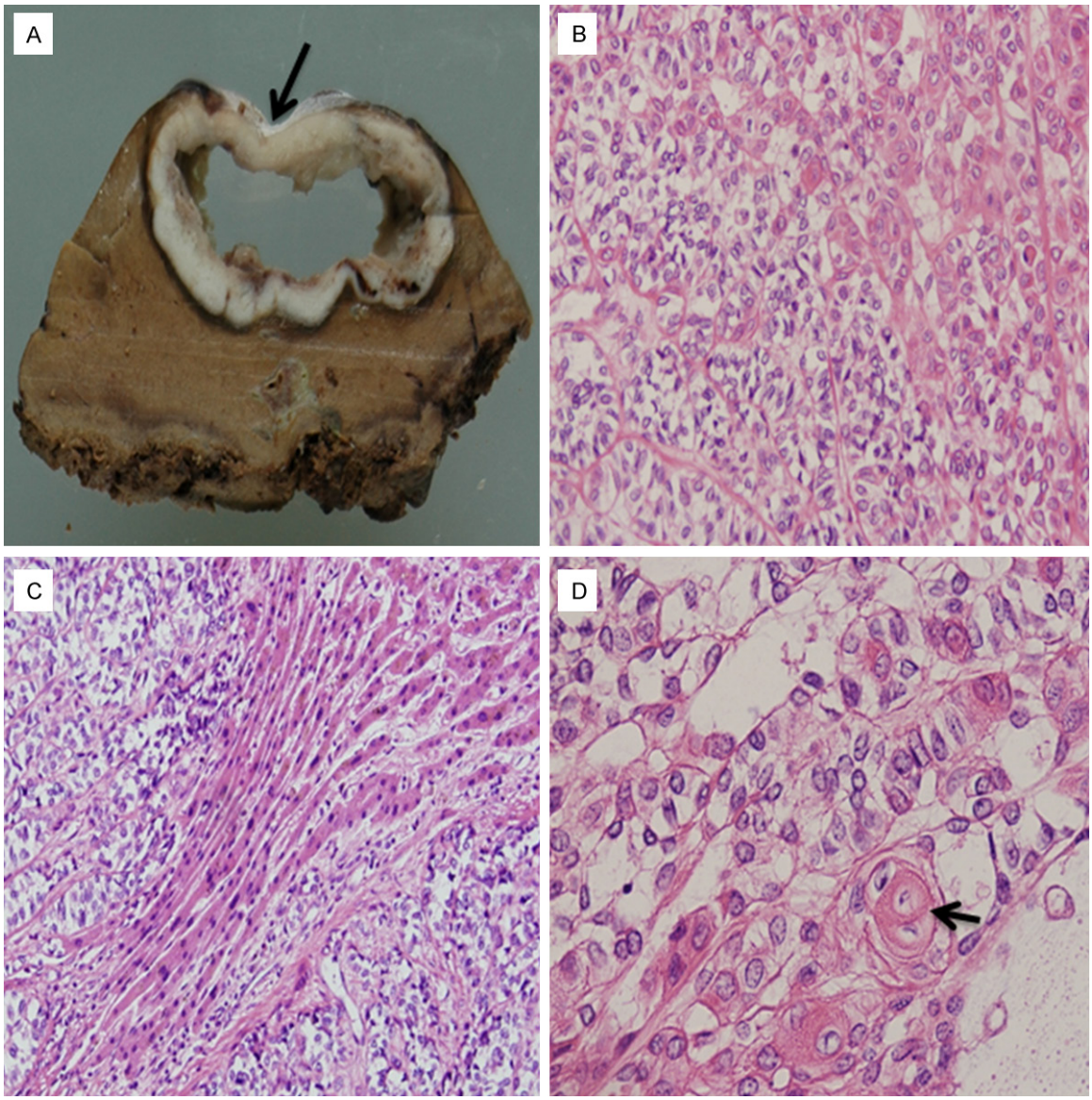

Figure 2. Gross and histologic features [hematoxylin & eosin stain] of liver tumor. A: A well-defined mass, 5 × 4 cm in size, with depressed outer surface [Arrow] and grey white cystic cut section. The rest of liver cut section shows no remarkable gross changes. B: Trabecular sheets and nests, formed from clear cells and esinophilic epithelioid cells (10 ×). C: Tumor cells infiltrate the surrounding non-neoplastic liver tissue (10 ×). D: The nests were formed from cells; some with clear cytoplasm and others with esinophilic cytoplasm. Focal presence of squamoid cells is seen (Arrow). Tumor cells have mild to moderate cellular and nuclear atypia, with slightly irregular nuclei and inconspicuous to small nucleoli (20 ×).

esinophilic granular reaction in cytoplasm after PAS staining, but not after diastase digestion, suggesting the presence of glycogen. The background liver showed mild lymphocytic infiltration in some portal tracts. By immunohistochemical staining, we first tried to confirm the possible origin of tumor cells using the following hepatocytic markers; AFP, HepPar-1, TTF-1 and glypican 3, and biliary markers; CK7, CK 19 and CEA. Immunostaining for the hepatocytic markers were all negative (**Figure 3A**). On the other hand, tumor cells showed cytoplasmic staining for CEA (**Figure 3B**), with focal areas showing cytoplasmic staining for CK7 and CK19, which probably indicate trapped bile ducts. We hypothesized that tumor cells were probably of biliary origin, as the cells showed no immunoreaction to all of the hepatocytic markers used, but instead, showed reaction to CEA, which might be explained by the biliary origin of

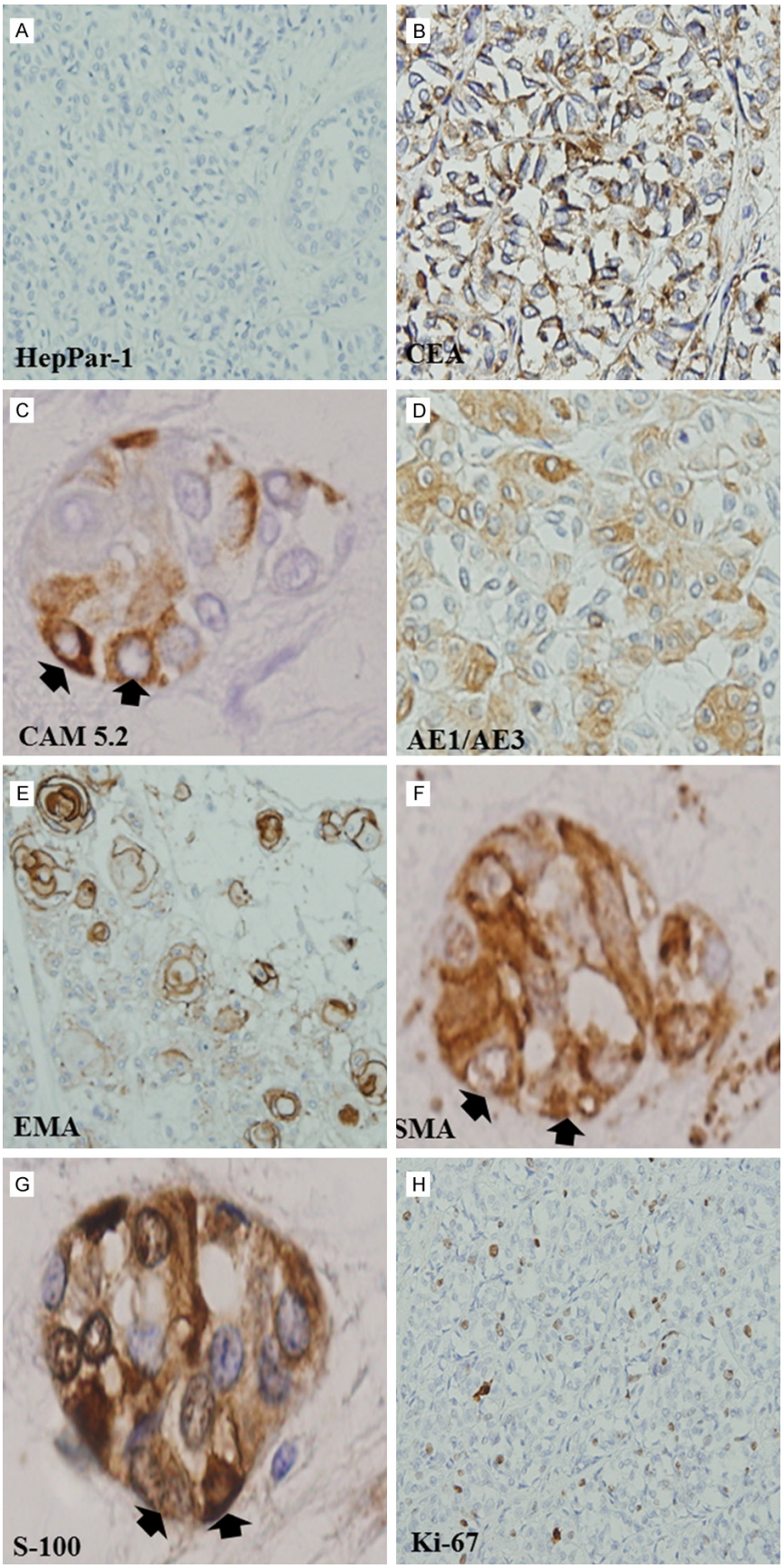

Figure 3. Results of immunohistochemical stains. (A) Negative staining for hepatocytic marker; HepPar-1 (10 ×). (B) Diffuse cytoplasmic staining for CEA , raising the possibility of biliary origin of the cells (20 ×). (C) Focal cytoplasmic staining for CAM 5.2 (Arrows) (40 ×). (D) Cytoplasmic staining of epithelioid cells for AE1/AE3 (20 ×). (E) EMA decorating squamoid cells (20 ×). (F and G) Serial sectioning revealed that cells [Arrows] that were positive for CAM 5.2 (C) stained for SMA and S-100, indicating the myoepithelial nature of the cells (40 ×). (H) 10-20% of tumor cells express mild to moderate Ki-67 nuclear staining (10 ×).

cells. Second we identified the nature of tumor cells by using antibodies to well-known epithelial and mesenchymal markers; CK14, CAM 5.2, AE1/AE3 and EMA as epithelial markers and vimentin, SMA, CD10, desmin, S-100, GFAP, p63 and calponin as mesenchymal markers. Tumor cells were negative for CK14, but showed focal cytoplasmic positivity for CAM 5.2 (**Figure 3C**). Epithelioid cells reacted preferentially to AE1/AE3 (**Figure 3D**) while squamoid cells were decorated with EMA (**Figure 3E**). Moreover, cells showed mild to moderate immunoreactivity to all myoepithelial markers used,

except calponin. Vimentin, SMA (**Figure 3F**), CD10, desmin and GFAP were seen positive in the cytoplasm of cells. S-100 is highly expressed in both the cytoplasm and nuclei of cells (**Figure 3G**), while p63 was seen occasionally positive in the nuclei of cells. We concluded that the tumor cells were of myoepithelial nature, due to their positive reaction to all myoepithelial markers used (except calponin). Moreover, antibodies to AE1/AE3 and EMA were previously reported to stain myoepithelial cells [10]. Regarding CAM 5.2, cells which showed positive reaction to it were stained with mesenchymal markers, SMA and S-100 (**Figure 3F** and **3G**) which emphasize on the myoepithelial nature of the cells. We hypothesized that such positive reaction to CAM 5.2 could be attributed to biliary origin of the tumor cells. Mitosis was rare and approximately 10-20% of tumor cells expressed mild to moderate nuclear ki-67 staining (**Figure 3H**). No atypical mitotic figures were seen. The results of immunochemical staining are summarized in **Table 1**. Based on these findings, the final histological diagnosis was myoepithelial carcinoma of the liver, most probably of biliary origin.

One year later, follow up examination with MRI, revealed two recurrent tumors in the left lobe of the liver, and partial left lobe lobectomy was performed. The tumor resected showed similar histology to the primary tumor. Three months later, another two recurrent tumors in the left lobe of the liver were detected by MRI, for which radiofrequency ablation was performed.

Discussion

Benign and malignant neoplasms of myoepithelial cells comprise a rare, but well-characterized group of tumors, that demonstrate a wide variation of cellular morphology and architectural pattern, due to the admixture of epithelial tumor cells and the expression of a variety of histological and immunohistochemical phenotypes by the neoplastic myoepithelial cells [11]. The diagnosis of myoepithelial tumor requires the presence of a pure population of myoepithelial cells [1, 12], whereas some authors have suggested the presence of up to 5% to 10% of epithelial ducts within the tumor [13, 14]. Myoepithelial cells may adopt a number of different morphologies including spindle cell, clear cell, epithelioid, and plasmacytoid cell forms [15]. The present case shows a mixture of spindle clear cells and epithelioid esinophilic cells. To our knowledge, there is no case reporting a primary myoepithelial carcinoma in liver. Only two case reports of epithelial myoepithelial neoplasm have been reported in liver [16, 17]. In both cases, the tumor was composed of duct-like structures with an inner layer of an epithelial lining, and an outer layer of clear cells; positive for myoepithelial markers. Regarding the present case, clinical examination and investigational work up showed no detected masses all over the patient's body, except in the liver, which led us to the assumption of a primary liver tumor. Moreover, the present case doesn't show the characteristic duct-like structures with double cell lining of the epithelial myoepithelial carcinoma, but shows trabecular sheets and nests composed of clear cells and esinophilic cells, that are stained with myoepithelial markers. In addition, we showed by a panel of immunohistochemical markers, that the tumor cells don't stain with hepatocytic markers, but instead stain with CEA, which might indicate the biliary origin of the tumor cells. Previous reports demonstrated that antibodies against calponin, S-100, GFAP and SMA have been successful in decorating the myoepithelial nature of the tumor [17]. Moreover, CAM 5.2 and CK14 were suggested as markers for epithelial nature of the tumor [8, 9]. Regarding AE1/AE3 and EMA, it was reported that these epithelial markers stain also myoepithelial cells [10]. In the present case, the tumor cells show positive staining for all the myoepithelial markers used [except for calponin]. No immunoreaction for CK14 was seen in tumor cells. Although the cells show positive reaction for CAM 5.2, yet the cells are seen to stain with myoepithelial markers; S-100 and SMA, indicating that these cells are of myoepithelial origin. We hypothesized that such immunoreactivity to CAM 5.2 might be attributed to biliary origin of the tumor cells. In addition, the detected squamoid cells in the present case have been described before in myoepithelial carcinoma [10]. Another salivary gland-type tumor that can occur in the liver is mucoepidermoid carcinoma which is regarded as a variant of intrahepatic cholangiocarcinoma [18]. Despite the presence of squamoid cells in the present case, yet the absence of mucin secreting glands rules out the possibility of mucoepidermoid carcinoma.

Most of salivary gland myoepithelial tumors have indolent clinical courses [8]. Defining criteria for malignancy in myoepithelial tumors include severe nuclear atypia, tumor infiltration of surrounding tissues, increased mitotic rate, and/or tumor necrosis [1, 2]. In the present case, despite that the tumor cells displayed mild to moderate nuclear atypia and low mitotic activity, yet the presence of focal infiltration into surrounding liver tissue, the tumor necrosis and the repeated recurrence of the tumor, indicate the high malignant potential of such tumor.

Disclosure of conflict of interest

The authors declare that they have no conflict of interest.

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