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Original contribution

Expression of glypican-3 in undifferentiated embryonal sarcoma and mesenchymal hamartoma of the liver☆

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Summary Glypican-3 (GPC3) is an oncofetal protein that has been demonstrated to be a useful diagnostic immunomarker for hepatocellular carcinoma and hepatoblastoma. Its expression in mesenchymal tumors of the liver, particularly undifferentiated embryonal sarcoma (UES) and mesenchymal hamartoma (MH), has not been investigated. In this study, a total of 24 UESs and 18 MHs were immunohistochemically stained for GPC3 expression. The results showed cytoplasmic staining for GPC3 in 14 (58%) UESs, of which 6 exhibited diffuse immunoreactivity and the remaining 8 showed focal positivity. The patients with GPC3-positive UES tended to be younger (mean 18 years; median 11 years) than those with GPC3-negative tumors (mean 39.4 years; median 27 years), although the difference did not reach statistical significance (P = .06). Eight MHs also exhibited GPC3 immunoreactivity (44%; 4 diffuse and 4 focal). Positive staining in all 8 cases was primarily seen in entrapped nonlesional hepatocytes with a canalicular and cytoplasmic staining pattern. In only 4 cases (22%) was GPC3 immunoreactivity also observed in the mesenchymal component. The patients with positive staining also tended to be younger (mean 2.6 years; median 1.1 years) compared with those with negative staining (mean 16.3 years; median 4.5 years), but the difference was not statistically significant (P = .15). Our data demonstrate that GPC3 is expressed in a subset of UES and MH of the liver. Caution should thus be exercised when evaluating a GPC3-expressing hepatic neoplasm,

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particularly on a needle biopsy when the differential diagnosis includes poorly differentiated hepatocellular carcinoma or hepatoblastoma.

1. Introduction

Mesenchymal hamartoma (MH) is an uncommon benign hepatic tumor primarily affecting the pediatric population within the first 2 years of life. Uncommonly, this tumor is seen in older children and adults [1-4]. It encompasses approximately 8% of all primary pediatric hepatic tumors and is the second most common primary hepatic tumor after hepatoblastoma. These tumors can be solid or cystic and quite large. The common histological features include a biphasic growth pattern composed of bland myxomatous stroma intertwined with branching duct structures, reminiscent of ductal plate malformation. Residual benign hepatocytes may be seen intermixed within the tumor but are more commonly noted at the periphery of the lesion. Malignant transformation to undifferentiated embryonal sarcoma (UES) has been reported but is rare [1,5,6].

On the other hand, UES is a malignant hepatic tumor with aggressive behavior. It primarily affects children between 2 and 5 years old and young adults. Rare cases have been reported in middle-aged and older patients [7-10]. These tumors are typically large, hemorrhagic, necrotic, and can be solid or cystic. Histologically, these tumors usually show proliferation of pleomorphic spindle cells in a myxoid stroma, which may surround or entrap benign bile ducts at the periphery. Intracellular and extracellular periodic acid–Schiff-positive hyaline globules may be seen [7,8,11]. Occasionally, UES may exhibit clinical, radiographic, or even histological features overlapping with hepatocellular carcinoma, hepatoblastoma, or MH, which makes the diagnosis difficult in a small biopsy specimen. In this regard, ancillary immunohistochemical studies may serve an important role in helping establish the diagnosis.

Glypican-3 (GPC3) is a member of the heparan sulfate proteoglycan family, which is linked to the cell surface through a glycosylphosphatidylinositol anchor [12]. This oncofetal protein is widely expressed in fetal tissues and is involved in organogenesis and growth control during development. In adults, GPC3 is exclusively expressed in neoplastic processes, notably hepatocellular carcinoma, hepatoblastoma, germ cell tumors, and Wilms tumor [13-18]. Studies have shown that serum GPC3 can be detected in 40% to 53% of patients with hepatocellular carcinoma, including a subset of patients that are seronegative for α-fetoprotein [19]. Immunohistochemical studies further demonstrate GPC3 to be a useful diagnostic immunomarker for hepatocellular carcinoma and hepatoblastoma, as it shows at least focal immunoreactivity in the majority of cases evaluated whereas benign liver lesions are typically negative for GPC3 expression [18,20-25]. However, the expression of GPC3 in nonvascular mesenchymal tumors of the liver, particularly UES and MH, has not been investigated.

2. Materials and methods

A total of 42 liver resection specimens were included in this study. These included 24 cases of UES and 18 cases of MH retrieved from surgical pathology archives of authors' institutions. The clinical history, pathology reports, hematoxylin and eosin–stained slides, and various diagnostic immunohistochemical stains were reviewed by the submitting pathologists to confirm the diagnosis. The study was approved by the Institutional Review Board at Cedars-Sinai Medical Center and coauthors' institutions.

Immunohistochemical staining for GPC3, α-fetoprotein, and hepatocyte antigen (Hep Par 1) was performed on all 42 cases. Formalin-fixed, paraffin-embedded tissue sections (4 μm) were deparaffinized, rehydrated, and treated with 3% hydrogen peroxide for 15 minutes to quench endogenous peroxidase. For GPC3 and Hep Par 1 immunostains, heat-induced epitope retrieval in 0.1 M citrate buffer at pH 6.0 for 20 minutes was performed. Antigen retrieval was not required for anti–α-fetoprotein antibody. The slides were incubated with a mouse monoclonal antibody (clone 1G12) specific for GPC3 obtained from BioMosaics (Burlington, VT), a rabbit polyclonal antibody specific for α-fetoprotein obtained from Dako (Carpinteria, CA), and a mouse monoclonal antibody (clone OCH1E5) specific for hepatocyte antigen obtained from Dako at room temperature at dilutions of 1:200 for 1 hour, 1:500 for 30 minutes, and 1:200 for 30 minutes, respectively. After incubation with an anti-mouse or anti-rabbit secondary antibody, a reaction was performed using the EnVision+ detection system that contained biotin-free horseradish peroxidase–labeled polymers obtained from Dako. The staining was visualized using 3,3′-diaminobenzidine substrate-chromogen solution and counterstained with hematoxylin.

Immunohistochemically stained slides were evaluated and a case was considered negative if less than 1% of the cells of interest exhibited immunoreactivity. Those cases with positive staining were graded as weak, intermediate, or strong for staining intensity. The percentage of positively stained cells in each case was also recorded. In general, the staining was considered focal if 1% to 50% of the cells of interest were stained and diffuse if more than 50% of the cells stained. Staining pattern (canalicular or cytoplasmic) was also recorded.