Immunocytological Differentiation of Hepatocellular Carcinoma versus Extragonadal Germ Cell Tumor

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Key Words

Extragonadal germ cell tumor
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Summary

Extragonadal germ cell tumor versus hepatocellular carcinoma must be distinguished in case of diffuse metastatic liver disease and highly elevated alpha-fetoprotein serum levels. The differential diagnosis is difficult, and conventional histological staining methods usually yield no results. However, liver puncture and immunocytology are new and reliable means for differentiation. The antibody HEA 125 reacts with all epithelial tumors including germ cell tumors, but shows no reactivity with normal or malignant hepatocytes. The practical value of this method is exemplified by the case of a 20-year-old patient with diffuse metastatic liver occupation by an extragonadal germ cell tumor.

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Zusammenfassung und Schlüsselwörter

Werden bei einem diffusen tumorösen Leberbefall stark erhöhte AFP-Spiegel festgestellt, so muß ein metastasierender extragonadaler Keimzelltumor – eine therapeutisch angehbare Neoplasie – von einem Leberzellkarzinom abgegrenzt werden. Während die Auflösung dieser Differentialdiagnose mit konventioneller Histologie häufig nicht möglich ist, kann dieses Problem mit Hilfe des HEA 125-Antikörpers immunzytologisch gelöst werden. Dies wird am Beispiel eines 20jährigen Patienten dargestellt.

Extragonadaler Keimzelltumor · Leberzellkarzinom ■ Immunhisto-chemie

Introduction

Extragonadal germ cell tumors are frequently accompanied by diffuse metastatic liver occupation. Especially in the case of highly elevated alpha-fetoprotein (AFP), it is hard to distinguish whether this tumor or a hepatocellular carcinoma is the cause of the metastatic liver disease, since both neoplasms are able to produce AFP. While conventional histology of liver tissue usually allows no differentiation between the tumors, this can be achieved using the antibody HEA 125 in immuno-histochemistry. This will be exemplified by the case of a 20-year-old patient.

Patient and Methods

A 20-year-old man with abdominal pain was found to have diffuse metastatic liver disease, diagnosed by ultrasound. His serum AFP-level (145,705 µg/l) was highly elevated, β-HCG in the physiological range. After immunocytological diagnosis of an extragonadal germ cell tumor, the patient was treated according to the PEB-regimen (Cisplatin 20 mg/m² i.v. V^{1/4} h, day 1–5; Etoposid 100 mg/m² i.v. 1 h, day 1–5; Bleomycine 30 mg i.v., day 1,8 and 15, rep. day 22). A continuous decrease of all tumor parameters resulted and after four cycles of therapy, AFP was in normal range (12.8 µg/l). After two more treatments were added, complete remission was achieved. Now, 18 months after therapy, the patient is still in complete remission. Liver puncture was performed in typical fashion with a Menghini-needle. The liver tissue was spreaded on glass slides and dried at room temperature. Before staining the slides were fixated in acetone for five minutes (4°C), air-dried for ten minutes and rehydrated in PBS-buffer solution (pH 7.4, ten minutes). For staining, the monoclonal antibody HEA 125 (E 022 – Histoprime, Camon/Wiesbaden, FRG) and the AFP-antibody (E 015, Camon) were used. The slides were incubated at room temperature for 60 minutes with the antibody-solution, then washed for 2 × 5 minutes in FKS/PBS, and afterwards treated with biotinyne-labeled anti-mouse IgG for 30 minutes. After washing in PBS for 20 minutes, the endogenous peroxidase was deactivated by incubation with peroxidase-labeled avidin-biotin-complex. Diaminobenzidine was used as a chromogene. The counterstaining followed with 'Mayer's Hämalaun', embedding with 'Kaiser's Glycerine Jelly'. The staining-process was performed on the immunohistochemical workbench Promedeus 10. Only 20 µl of primary antibody had to be used.

Results

The conventional histology of the Menghini-puncture showed parts of a solid tumor with large, partly anaplastic cells and high mitotic activity. The clinically relevant differential diagnosis of secondary disseminated liver occupation by an extragonadal germ cell tumor versus hepatocellular carcinoma could not be solved by these means. In cytology with Pappenheim-staining, tumor cells could be detected (fig. 1). Their immunocytological reaction with HEA 125 antibody was positive (fig. 2), likewise with AFP-antibody.

Discussion

Differential diagnosis of hepatocellular carcinoma versus secondary disseminated metastatic liver disease by means of conventional histology frequently poses problems, especially in the case of an extragonadal germ cell tumor, since this neo-

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Fig. 1. Cytological smear of liver-biopsy, Pappenheimstain: Mass of an extragonadal germ cell tumor (x 500)

Fig. 2. Cytological smear of liver-biopsy, Immunocytology: HEA 125 positive cells of an extragonadal germ cell tumor. Below right: HEA 125 negative hepatocytes (ABC-PO, x 500). plasm produces AFP as well and is nearly impossible to find. It is of great practical importance, however, to find a solution to this problem, since extragonadal germ cell tumors are potentially cureable, while the hepatocellular carcinoma has no prognosis.

According to our experience, the HEA 125 antibody provides a secure tool to solve the differential diagnosis. This glycoprotein-antibody reacts specifically with epithelial structures including germ cell tumors [1, 3–5]. It has no affinity to normal or malignant hepatocytes, however, shown by an immunohistochemical study with ten cases of hepatocellular carcinoma [2]. Thus, in our case with highly elevated AFP serum levels and the positive HEA 125 tissue

reactivity, the diagnosis of an extragonadal germ cell tumor was evident. Conclusively, in our opinion the combination of liver puncture and immunocytological or immunohistological staining with HEA 125 appears to be a valuable and safe method to distinguish between hepatocellular carcinoma and extragonadal germ cell tumor in cases of diffuse metastatic liver disease and elevated AFP levels.

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