LETTER TO THE EDITOR

α-Fetoprotein Immunoreactivity in Human Choroid Plexus

Alpha-fetoprotein (AFP) is a major serum protein during human fetal development, because it is synthesized predominantly by fetal liver cells, yolk sac cells and in trace amounts by fetal gastrointestinal epithelial cells (9). The production of AFP ceases after maturation, except in neoplasms such as hepatocellular carcinoma or teratocarcinoma (8). AFP is thus used as a tumor marker in a clinical setting (8). The presence of a trace amount of AFP (less than 5 ng/ml) has been reported in the serum in adults (5). However, there have been no reports on the site of origin of AFP production in human adults.

We describe the immunohistochemical localization of AFP in human choroid plexus that was demonstrated by using four different kinds of antibodies against AFP.

Paraffin sections of formalin-fixed autopsied human brains were used for this study. Twelve cases were chosen from a series of autopsies performed at Kyoto Prefectural University of Medicine Hospital. Table 1 shows the clinical and pathological diagnoses of the patients examined. Six of them showed no notable changes neuropathologically, whereas the other six showed some changes in the brain parenchyma. None of the patients studied showed high titers of serum AFP.

Paraffin sections 4 μm in thickness including the choroid plexus present in the lateral as well as the fourth ventricle were processed for immunohistochemistry for α-fetoprotein by the avidin-biotin horseradish peroxidase complex (ABC) technique. The antibodies used were one polyclonal (rabbit, DAKO, Denmark; dilution 1 : 20-200), and three monoclonals (mouse IgG1, kappa: one was purchased from Cosmo Bio and the other two from Tokushumen’eki Laboratory, Japan; dilution 1 : 100-200 for the former, 1 : 20-200 for the latter two). The latter two were reported to recognize different epitopes present in AFP.

The sections were incubated with 3% H₂O₂ in methanol for 30 min in order to block the endogenous peroxidase activity, and followed by incubation with either normal goat serum (when using polyclonal antibody as a primary antibody) or normal horse serum (when using monoclonal antibodies as primary antibodies) for 20 min to block nonspecific binding. The sections were incubated thereafter with one of the antibodies against AFP either for one hr at room temperature (for polyclonal) or overnight at 4°C (for three monoclonals). After rinsing with 0.01 M phosphate buffered saline (PBS, pH 7.2), biotinylated goat anti-rabbit IgG or horse anti-mouse IgG was applied at the dilution of 1 : 200 for 30 min at room temperature. After rinsing with PBS they were incubated with avidin-biotin peroxidase complex (Vectastain kit, Vector Laboratories, USA) for 30 min at room temperature. The color reaction was performed by incubating the sections with a mixture of 0.02% 3,3′-diaminobenzidine 4HCl (DAB, Dainippon, Japan) and 0.005% H₂O₂ for 5–15 min. The sections were counterstained either with methyl green or with hematoxylin.

All the sections revealed the AFP immunoreactivity in the cytoplasm of the choroid plexus epithelial cells (Fig. 1). The nuclei were not stained with any of the antibodies. Neurons, glial cells and ependymal cells were also not stained. Human hepatocellular carcinoma cells that were used as positive controls were stained with all the four antibodies studied. In the negative controls where, instead of the primary antibody against AFP, normal rabbit serum, anti-human C1q (polyclonal, rabbit Ig, DAKO), anti-glial fibrillary acidic protein (GFAP, polyclonal, rabbit Ig, DAKO), anti-human leukocyte common antigen (CD45, monoclonal, mouse IgG1, DAKO), and PBS were used, no immunoreactivity was detected in the choroid plexus epithelium.

The choroid plexus is known to be involved in the formation and homeostasis of the cerebrospinal fluid (1, 11). Prealbumin (4, 6, 13), transferrin (2, 6) and
TABLE 1. Clinical and pathological diagnoses of patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Autopsy diagnosis</th>
<th>Age (yrs)</th>
<th>Sex</th>
<th>Neuropathological findings</th>
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<tr>
<td>1</td>
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<td>75</td>
<td>F</td>
<td>not remarkable</td>
</tr>
<tr>
<td>2</td>
<td>hypertrophic cardiomyopathy</td>
<td>54</td>
<td>M</td>
<td>not remarkable</td>
</tr>
<tr>
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<td>neuroblastoma</td>
<td>9</td>
<td>F</td>
<td>not remarkable</td>
</tr>
<tr>
<td>4</td>
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<td>65</td>
<td>M</td>
<td>not remarkable</td>
</tr>
<tr>
<td>5</td>
<td>schizophrenia</td>
<td>43</td>
<td>M</td>
<td>not remarkable</td>
</tr>
<tr>
<td>6</td>
<td>chronic subdural hematoma</td>
<td>76</td>
<td>M</td>
<td>not remarkable</td>
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<tr>
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<td>M</td>
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<td>57</td>
<td>F</td>
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<td>F</td>
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<tr>
<td>12</td>
<td>glioblastoma</td>
<td>74</td>
<td>M</td>
<td>glioblastoma</td>
</tr>
</tbody>
</table>

M: male, F: female, SLE: systemic lupus erythematosus, CNS: central nervous system

**Fig. 1. Immunohistochemistry for AFP.**

Immunoreactivity for AFP is detected as granular reaction products in the choroid plexus epithelial cells. Similar staining was present in all areas of the choroid plexus epithelium. Stromal and nuclei were negative. Counterstaining with methyl green. Scale bar = 20 μm. Primary antibody, monoclonal anti-human AFP (Cosmo Bio). Subject 1 in Table 1.
ceruloplasmin (3) have been reported to be synthesized in the choroid plexus epithelium.

Prealbumin is involved in the transport of thyroid hormones (7) and retinol (14) in serum. Transferrin transports iron, whereas ceruloplasmin transports copper in the serum. However, the functional roles of these three proteins in the choroid plexus remain to be elucidated. These proteins including AFP are all serum proteins that are synthesized principally in the liver, and can be regarded as "brain-gut peptides" (12). Similar peptides such as cholecystokinin and gastrin, exist in both brain parenchyma and gut, although they may play different functional roles depending on the organs where they are present, namely as neuromodulators in the brain and as hormones in the gut. In this context a novel concept "brain-liver proteins" may be proposed based upon our finding on AFP in conjunction with previous reports by others on the presence of prealbumin (4, 6, 13), transferrin (2, 6) and ceruloplasmin (3). However, it should be noted that positive immunoreactivity for AFP shown here does not imply synthesis of AFP in the choroid plexus epithelium. There are at least two interpretations for our observation on the AFP immunoreactivity in the choroid plexus epithelium: one is absorption of AFP that was synthesized elsewhere, and the other is de novo synthesis of the protein. In situ hybridization for the AFP mRNA (10) could clarify this issue. Another question is whether AFP detected immunohistochemically in the choroid plexus is the same as that found in the fetal cells. In other words, an unidentified protein(s) may be incidentally detected by the antibody which we used. However, this is unlikely, because in our study all the antibodies including monoclonals that recognize different epitopes of AFP showed a similar pattern of positive staining in the choroid plexus epithelium. To confirm further whether authentic AFP is present in the choroid plexus epithelium, immunoblot studies are needed.

A trace amount of AFP has been detected in normal human adult serum (5), despite the fact that AFP is not detectable in the adult human tissue except in the choroid plexus as shown here. Thus the choroid plexus might play a role as a source for providing a trace amount of AFP in the serum in adults.

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REFERENCES

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SHINJI FUSHIKI*, HIROTO YAMAZAKI* and SETSUUYA FUJITA**

*Department of Dynamic Pathology, Research Institute for Neurological Diseases and Geriatrics, and **Department of Pathology, Kyoto Prefectural University of Medicine, Kawaramachi Hirokoji, Kamigyo-ku, Kyoto 602