Short communication

Expression of stanniocalcin in the epithelium of human choroid plexus

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Abstract

Stanniocalcin (STC) is a 28 kD glycoprotein hormone originally found in bony fish in which it regulates calcium/phosphate homeostasis and protects against hypercalcemia. The recently characterized mammalian STC shows about 70% homology with fish STC. The epithelial cells of proximal tubuli in human and rat kidney and brain neurons have been found to express STC. Here we show that the epithelium of the choroid plexus, already at 16 weeks of fetal age, and of plexus papillomas, synthesize and express STC. Our findings suggest that STC may be of importance for the distribution of calcium and phosphate between the cerebrospinal fluid and blood.

Theme: Cellular and molecular biology
Topic: Staining, tracing, and imaging techniques
Keywords: Stanniocalcin; Blood–brain barrier; Phosphate homeostasis; Calcium transport

The choroid plexus (CP) is a specialized secretory organ, which produces cerebrospinal fluid (CSF). CP consists of invaginated fronds of leptominges around a highly vascularized, fibrous stroma. The plexus is covered by an ependyma-derived epithelium forming a single layer of polarized, cuboidal cells.

Primary neoplasms originating in the epithelium of the CP are rare. They mostly consist of benign papillomas, which occur both in childhood, and at adult age. Usually they come to attention due to their propensity of obstructing CSF circulation or producing excess CSF. Malignant tumors derived from the CP, i.e. carcinomas, are exceedingly rare and occur mostly in infancy or childhood.

Stanniocalcin (STC) is a 28 KD glycoprotein originally discovered in fish [23]. Fish STC is synthesized in a specialized organ, the Corpuscle of Stannius which is located adjacent to the kidney [16]. STC plays a major role in regulating the calcium/phosphate homeostasis in fish. It protects against hypercalcemia by lowering the uptake of calcium via the gills [7,10] and the intestinal tract [18] and increases reabsorption of phosphate in the kidney [11]. Elevated environmental concentration of calcium is the main trigger of STC production in fish [20,21]. STC was considered exclusive to fish until the cDNAs for human [3] and mouse [4] STC were cloned.

STC has remained highly conserved during evolution. Human STC cDNA shares a 72% nucleotide sequence homology with fish STC and the protein shows 80% similarity with conserved cysteins [3]. The ultimate function(s) of STC in mammalians remains to be clarified but it appears to regulate the phosphate/calcium balance. Intravenous infusion of recombinant human STC to rats was found to increase the reabsorption of phosphate in the kidney [22]. Histochemical investigations have revealed a constitutive expression of STC in the epithelial cells of the proximal tubuli in human [6], rat [8,24] and mouse kidney [25]. Expression of STC mRNA has been found in various tissues, including the heart muscle, prostate and gastrointestinal tract [3].

We originally reported a high expression of STC in...
terminally differentiated neurons in mouse and human brain [26]. Glial cells did not express detectable levels of STC but we observed a strong positive immunohistochemically stained of the epithelial cells of the CP.

In this study we show that the epithelium of CP constitutively synthesizes and expresses STC. The neoplastic epithelium of CP papillomas was also found to express STC.

Biopsies of CP from ten patients, five with papilloma and five with miscellaneous diseases, were studied. In addition, CP tissue was obtained from autopsies of two fetuses at 16 and 21 weeks of gestation age (Table 1). The tissue was fixed in 4% buffered formaldehyde for 12–48 h, routinely processed and embedded in paraffin. Four µm thick sections were mounted on 3-aminopropyl-triethoxysilane (APES) (Sigma, St. Louis, MO, USA) coated slides and dried for 12 h at 37°C. Immunohistochemical stainings of deparaffinized and rehydrated sections with rabbit antibodies to human STC [26] were performed by using a commercial Elite ABC Kit (Vectastain, Vector Laboratories, Burlingame, CA, USA) as described in detail [26]. Antibodies to STC pre-absorbed with recombinant STC (Proteins, Burlingame, CA, USA) as described in detail [26].

Table 1 Choroid plexus tissue used in the study

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>Reason for CP resection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2/M</td>
<td>Papilloma</td>
</tr>
<tr>
<td>2</td>
<td>35/M</td>
<td>Papilloma</td>
</tr>
<tr>
<td>3</td>
<td>59/F</td>
<td>Papilloma</td>
</tr>
<tr>
<td>4</td>
<td>62/F</td>
<td>Papilloma</td>
</tr>
<tr>
<td>5</td>
<td>65/F</td>
<td>Papilloma</td>
</tr>
<tr>
<td>6</td>
<td>1/F</td>
<td>Meningeal angiomatosis</td>
</tr>
<tr>
<td>7</td>
<td>31/M</td>
<td>Astrocytoma, G2</td>
</tr>
<tr>
<td>8</td>
<td>49/M</td>
<td>Meningeoma</td>
</tr>
<tr>
<td>9</td>
<td>55/F</td>
<td>Metastatic melanoma</td>
</tr>
<tr>
<td>10</td>
<td>60/F</td>
<td>Metastatic breast cancer</td>
</tr>
<tr>
<td>11</td>
<td>Fetus 16 weeks of GA</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>Fetus 21 weeks of GA</td>
<td></td>
</tr>
</tbody>
</table>

To study whether the STC in the epithelium of CP is synthesized locally or taken up from the circulation we performed in situ hybridization. The anti-sense single-strand RNA probe for STC gave a strong reactivity confined to the epithelial cells (Fig. 1c) while no specific reactivity was obtained with the control (sense) probe (Fig. 1d).

When five cases of CP papillomas were immunohistochemically stained, a similar expression of STC in the neoplastic epithelium was seen in all cases although the staining intensity showed more variation than in the normal CP epithelium (Fig. 1f).

The epithelial cells of CP localize in between the blood and CSF. Circulating STC (4.5 to 11.6 ng/ml) has been found in human serum [19]. We used an STC immunoassay to measure the amount of STC in the CSF collected at the Department of Occupational Health for diagnostic purposes from five adult persons suffering from headache with no known organic cause. All samples contained less than 2 ng/ml STC (the detection limit of our assay, data not shown).

The CP not only produces the CSF, but it also constitutes an essential part of the blood-CSF barrier. In this localization, the epithelial cells of the CP are actively regulating the distribution of molecules between the blood and CSF. In this respect the epithelium shares many properties with renal tubular cells. The CP has been called a ‘kidney’ in the brain with the difference, however, that the kidney clears waste products from the blood while the CP transports waste products from CSF into the blood [15].

Co-expression of a variety of proteins involved in epithelial barrier functions has been found in CP and kidney tubular cells. These include isoforms of the plasma membrane Ca2+ pump [17], the anionic transport protein [1], the angiotensin-converting enzyme [5], the Na+K+ATPase [14], and the recently characterized inwardly rectifying K+ channel [13]. This study adds STC to the list.

Given the analogous transport functions, it is interesting that the epithelial cells of both the proximal tubuli of mammalian kidney and the epithelium of CP shown here express STC. The function of STC, regulating the calcium/phosphate homeostasis in fish, appears conserved through evolution. Addition of recombinant human STC to isolated pig duodenal epithelium increased the cross-epithelial transport of phosphate [12]. Recent studies indicate that the sodium dependent phosphate co-transporter (NaPi-2) is a target of action of STC in mammalian kidney [22]. Whether the NaPi is expressed also in the choroid plexus remains to be established. It is, however, tempting to speculate that STC in the choroid plexus may be involved in the regulation of the phosphate/calcium homeostasis in the CSF [2,9]. The local activity of STC may also have bearings on the pathogenesis of the calcified foci frequently found in papillomas of the CP.
Fig. 1. Immunohistochemical staining of normal CP with antibodies to human STC (a), and with STC antibodies preabsorbed with recombinant STC protein as a control (b). In situ hybridization with an *stc* anti-sense probe on normal CP (c), and with the sense probe as a control (d). Immunohistochemical staining of fetal CP (16 weeks) with antibodies to STC (e). Immunohistochemical staining of CP papilloma with STC antibodies (f). The scale bar represents 200 µm in (a), (b), (c), (d) and 100 µm in (e) and (f).

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References


