Abstract Suprasellar germinomas were identified in three wild-caught lake whitefish (*Coregonus clupeaformis*) from the St. Lawrence River, Quebec, Canada. Histologically, the three tumors expanded the subarachnoid space of the ventral surface of the brain immediately adjacent to the pituitary gland and, in one case, infiltrated the adjacent neuropil. These tumors were characterized by nests and sheets of round cells with a high mitotic rate, separated by a scant amount of loose fibrovascular stroma. The stroma was infiltrated by a moderate number of small mononuclear cells, including rare CD3-immunoreactive lymphocytes. This is the first report of intracranial germ cell tumor in a fish species.

Key words Germ cell tumor · Suprasellar · Fish Cancer

Introduction

Suprasellar germ cell tumors are neoplasms of gonadal germ cells which develop at the base of the skull cavity, right above the sella turcica. The current theory for the embryological origin of those tumors implicates abnormalities in germ cell migration during embryogenesis and their subsequent transformation into neoplastic cells [3]. The histogenesis and differentiation of extragonadal germ cell tumors are analogous to those of their gonadal counterparts: pure germ-line tumors are called germinomas, while differentiated germ cell tumors are divided into embryonic (embryonal carcinoma and mature or immature teratoma) and extraembryonic (choriocarcinoma and endodermal sinus tumor). Germinomas are histologically identical to testicular seminoma and ovarian dysgerminoma [19, 24].

In man, intracranial germ cell tumors are rare tumors of children and young adults. They are extremely rare in animal species and isolated cases have been described in dogs [23], cows, rabbits, and chicken [22]. Here we describe the histology and ultrastructure of germ cell tumors of the germinoma type in three lake whitefish (*Coregonus clupeaformis*), a North American species found in cold and temperate waters, from a population with high tissue concentrations of environmental contaminants (de Lafontaine, unpublished) and high prevalence of hepatic tumors [17].

Methods

Lake whitefish (*n* = 512) were collected using a fixed fishing gear installed at Saint-Nicholas (46 43’ N, 71 19’ W), Quebec, Canada, from 1 September 1996 to 30 October 1996. Fish were killed with an overdose of tricaine methane sulfonate (MS 222) and a complete postmortem examination was performed. Samples of major organs, including a transverse section of the brain through the pituitary, were preserved in 10% buffered formalin for histological examination. Samples were embedded in paraffin wax, sectioned at 4 μm and stained with hematoxylin-phloxin-saffron. Additional formalin-fixed specimens of two tumors were routinely processed.
for transmission electron microscopy. Serial sections of each tissue were immunostained for CD3, using an antibody which has been shown to stain lymphocytes in T-dependant areas in a broad range of species [7] (rabbit anti-human, polyclonal, 1:500; Dako Corp., Carpinteria, Calif.), cytokeratins (anti-human monoclonal, 1:50; Biocare Medical, Walnut Creek, Calif.), neuron-specific enolase (NSE; rabbit anti-human, polyclonal, 1:40; Biogenex, San Ramon, Calif.), estrogen-receptor (ER; rabbit anti-human, polyclonal, ready to use; Zymed Laboratories Inc., So. San Francisco, Calif.), alpha-fetoprotein (α-FP; rabbit anti-human, polyclonal, 1:5000; Dako), human chorionic gonadotrophin (HCG; rabbit anti-human, polyclonal, 1:4000; Dako), placent al alkaline phosphatase (PLAP; rabbit anti-human, polyclonal, 1:800; Dako), glial fibrillary acidic protein (GFAP; rabbit anti-bovine, polyclonal, 1:500; Dako), S-100 protein (rabbit anti-bovine, polyclonal, 1:10; Dako), synaptophysis (rabbit anti-human, polyclonal, 1:20; Dako), chromogranin (mouse anti-human, monoclonal, 1:40; Dako) antisera by the avidin-biotin-peroxidase complex (ABC) method. Sections of tumor were also stained under identical conditions with an irrelevant antibody to serve as negative controls (anti-porcine rotavirus, Institut Armand Frappier, Pointe-Claire, Quebec, Canada). Positive controls were gonads from whitefish for anti-ER antibody, anterior kidney from whitefish for anti-CD3 and anti-CLA antibodies, multiple whitefish epithelial tissues for anti-cytokeratins, and whitefish brain for anti-NSE, anti-GFAP, anti-NF, anti-S100, anti-synaptophysin, and anti-chromogranin antibodies, and positive human tumors for anti-HCG, anti-PLAP, and α-FP antibodies. All immunostained sections were counterstained with Mayer’s hematoxylin. Histological slides of the tumors were deposed in the Registry of Tumors in Lower Animals, George Washington University, Washington, D.C. (RTLA 6543–6545).

A Fisher exact test was used to compare the prevalence of lesions between sex and age groups. Statistical analyses were performed using the 6.12 version of the SAS software (SAS Institute Inc., Cary, North Carolina, USA).

Results

No macroscopic abnormality was found upon examination of the brain of 512 fish. Three germ cell tumors were diagnosed in two males and one female. These animals measured 409 mm, 430 mm, 458 mm and, therefore, were considered as young adults. Maturity in this lake whitefish population occurs at 339–447 mm (de Lafontaine, unpublished data).

The tumors were very similar among the three fish. They were located in the subarachnoid space at the base of the brain, surrounded the stalk of the pituitary gland, and extended dorsally and bilaterally in the subarachnoid space up to the mid-third (2 cases) to the mid-half (1 case) of the brain (Fig. 1). One of the tumors multifocally invad ed the neuropil of the optic lobe, dorsally to the pituitary. Tumors consisted of round cells arranged in small nests separated by a delicate, almost imperceptible fibrovascular stroma infiltrated by a few small lymphocyte-like cells (Fig. 2). Neoplastic cells were closely packed, monomorphic, large, round, with a small amount of finely granular pale eosinophilic cytoplasm. Their nucleus was central, round, large, hyperchromatic, with dense, finely stippled chromatin and faint to inapparent nucleolus. Mitoses were numerous and, in one case, about 10% of the neoplastic cells were mitotic.

Ultrastructural examination was hampered by poor preservation of the tissues attributed to prolonged storage in buffered formalin prior to preparation for ultrastructural examination. Neoplastic cells formed large islets that were not delimited by a basement membrane. Neoplastic cells were closely packed, ovoid, lacked junctional complexes and had interdigitating plasma membranes. The cytoplasm was scant and contained abundant glycogen granules and occasional long sheets of smooth endoplasmic reticulum, a Golgi apparatus and a few small mitochondria around the nucleus. Other organelles were not found. Nuclei were large, oval, with homogenous distribution of dense granular chromatin and one small nucleolus. The stroma contained rare lymphocytes and macrophages.

Sections of tumors and control fish tissues did not stain with the antibodies used, with the exception of the anti-CD3 antibody which stained a few stromal lymphoid-like cells of the tumors and of the kidney, and the anti-cytokeratin antibody which faintly stained epithelial tissues but not the tumor cells.

Infiltration of the peri-pituitary subarachnoid space by small numbers of loosely scattered small round cells with little cytoplasm was observed in 53 of 509 (10.4%) fish...