Spontaneous Malignant Craniopharyngioma in an Aged Wistar Rat

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Running title
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Abstract

Craniopharyngiomas are extremely rare epithelial tumors of the sellar region in human beings and domestic and laboratory animals. A craniopharyngioma, 0.6 cm in diameter, was observed grossly in the sellar and parasellar regions of an untreated 23-month-old male Wistar-derived rat sacrificed moribund. The tumor was composed of cords, columns, and nests of neoplastic stratified squamous epithelium with marked hyperkeratosis and parakeratosis. Neoplasic cells formed solid or cystic areas, infiltrating the base of the skull, brain, and pituitary gland. Immunocytochemical evaluation revealed a strong cytoplasmic reaction for keratin in all tumor cells. Malignant craniopharyngioma should be considered a differential diagnosis in the rat when a tumor with stratified squamous epithelial features and a locally aggressive growth pattern is observed in the sellar or suprasellar region.
Keywords: craniopharyngioma, malignant craniopharyngioma, cytokeratin, immunocytochemistry, pituitary tumor, rat
Craniopharyngiomas are rare epithelial tumors of the sellar region histogenetically derived from remnants of Rathke's pouch. In human beings, they represent approximately 2 to 3% of all intracranial tumors and are among the most common tumors of childhood and adolescence\(^1\). Clinical signs and symptoms vary with tumor size and location and include visual disturbance and endocrinologic abnormalities, e.g., growth retardation, diabetes insipidus, and hyperprolactinemia\(^1\). Craniopharyngiomas, extremely rare in animals, have been documented in a gerbil\(^2\) and a mouse\(^3\). Several cases have been reported also in dogs, but most of them have been reclassified as suprasellar germ cell tumors\(^4\). The same might apply to the two cases described in cats\(^5\). In the rat, several cases of craniopharyngioma have been reported\(^6-10\), but they have not been documented or described adequately or suffer from inconsistencies.

A 23-month-old male Wistar (Han:WIST)-derived rat was found apathetic and hypothermic. Because of its deteriorating general condition, it was euthanized, and a complete necropsy was performed. The rats had been kept alone in hanging wire-mesh top Makrolon cages on softwood bedding and maintained under conventional conditions in a temperature-controlled room (20-23°C) of the animal facility of the Institut für Veterinär-Pathologie, Justus-Liebig-Universität, Gießen, Germany, on a natural light cycle with standard rat chow pellets (Altromin 1320, Altromin, Lage, Germany) and tap water available ad libitum. Rat care was carried out in accordance with all applicable guidelines of the German Animal Welfare Act. The medical records revealed no previous disease or treatments of this rat.

Various tissues, including the tumor, brain, skull, lungs, liver, kidneys, adrenals, pancreas, duodenum, and testes with epididymides, were fixed in Bouin's solution and embedded in paraffin. Sections with thicknesses of 5 to 7 µm were mounted on gelatine-coated glass slides and stained with hematoxylin and eosin; when appropriate, Masson's trichrome or Klüver-Barrera's luxol fast blue cresyl violet stains were applied. For immunocytochemical staining, sections were processed according to the peroxidase-antiperoxidase (PAP) method or an alkaline phosphatase streptavidin-biotin labelling system. Rabbit antisera to the following antigens with their specified optimal dilutions were employed: rat prolactin (anti-rPRL, 19602,
1:1000, Dr. N. Martinat, Institut National de la Recherche Agronomique, Nouzilly, France), rat growth hormone (anti-rGH, 1:5000, UCB Bioproducts, Brussels, Belgium), β¹-²⁴-corticotropin (anti-ACTH, 81/2, 1:2250, Drs. S. Blähser and M. Heinrichs, Justus-Liebig-Universität, Gießen, Germany), rat luteinizing hormone β-subunit (anti-rLHß, AFP-2-11-27, 1:16000) and rat thyroid stimulating-hormone β-subunit (anti-rTSHß, AFP-1-9-15, 1:30000, both from Dr. A. F. Parlow, National Hormone and Pituitary Program, Baltimore, MD, USA), porcine neurophysin (anti-NPS, Rb 42, 1:2000, Dr. M. V. Sofroniew, University of Cambridge, Cambridge, United Kingdom), and glial fibrillary acidic protein (anti-GFAP, 1:1000, Dako, Hamburg, Germany). In addition, a mouse monoclonal antibody against pan-cytokeratin was used (Lu-5, 1:10, Boehringer Mannheim, Mannheim, Germany) after pretreatment of the sections with protease type XXIV (Sigma, Deisenhofen, Germany). Controls included substitution of primary antibodies with normal rabbit and mouse serum, respectively, or Tris-buffered saline, as well as omission of the second antibody or the PAP-complex.

Macroscopically, a tan-white mass, approximately 0.6 cm diameter, indenting the overlying brain was found paramedially in the sellar and suprasellar regions (Fig. 1), and it extended rostrally almost to the olfactory bulbs and caudally to the pons. The pituitary gland was not discernable from the mass. On the cut surface, the base of the skull appeared thickened and infiltrated by tumor tissue containing a few small cysts. The testes were markedly atrophic, and the lungs showed multiple miliary greyish spots on their dorsal surfaces. No additional gross lesions in other organs were encountered.

Microscopically, the tumor was composed of cords, columns, and nests of irregular stratified squamous epithelium with no keratohyaline granules, exhibiting marked hyperkeratosis and parakeratosis separated by a delicate fibrovascular stroma. Tumor cells either formed solid areas or, predominantly, cyst-like structures of varying size filled with desquamated keratin in somehow irregular layers or squames, cellular debris, macrophages, and/or proteinaceous fluid (Figs. 2 and 3). Tumor cells had hypochromatic vesicular nuclei with prominent nucleoli. Mitotic figures were frequently demonstrated (Fig. 3). Focally, there was mild neutrophil infiltration. The tumor had infiltrated the base of the skull, destroying most of the sphenoid
bone (Fig. 4). The pituitary gland was found adjacent to the medial margin of the tumor, mildly displaced beyond the midline due to tumor extension, with unilateral destruction of the intervening trigeminal ganglion. In addition, minimal focal infiltration of the pituitary pars distalis was present (Fig. 5). The pituitary pars intermedia and pars nervosa were unremarkable. Moreover, a single focus of invasion associated with focal demyelination was observed unilaterally in the ventrolateral telencephalon (Figs. 1 and 6). Microscopically, the focus was characterized by cords of neoplastic stratified squamous epithelium with no keratohyaline granules and little or no hyperkeratosis and parakeratosis. In the associated cystic focus of demyelination, many macrophages and neutrophils, cellular debris, and adjacent hemorrhage were evident (Fig. 6). There was no infiltration of the tumor into the nasal cavity, orbit, or skull at the base of the auricles. The histomorphologic features and the wide-spread infiltration described were consistent with the diagnosis of a malignant craniopharyngioma.

There was mild to moderate bilateral chronic progressive nephropathy. In the lungs, mild alveolar histiocytosis and mild multifocal suppurative bronchopneumonia were observed. Marked diffuse degeneration and atrophy of the germinal epithelium were conspicuous in both testes. All other tissues were unremarkable.

There was strong cytoplasmic staining of tumor cells and desquamated keratin for pan-cytokeratin (Fig. 4) in all parts of the malignant craniopharyngioma. Immunocytochemistry disclosed normal distributions and numbers of cells stained with antibodies against rPRL, rGH (Fig. 4), ACTH, rLHß, and rTSHß in the pituitary pars distalis. Moreover, immunostaining for NPS showed no abnormalities in the hypothalamic paraventricular and supraoptic nuclei and the pituitary pars nervosa. The neuropil was focally compressed by the cystic focus of demyelination and exhibited many reactive GFAP-positive astrocytes.

The histomorphologic features of the present tumor were consistent with the diagnosis of a craniopharyngioma occurring in aged rats. Moreover, the tumor infiltrated the base of the skull, brain, and pituitary gland, suggesting malignant behavior. Previous reports mentioned a similar or comparable locally aggressive growth pattern of the tumor, but avoided any
discussion on malignancy. The tumor was characterized by a neoplastic stratified squamous epithelium with no keratohyaline granules, marked hyperkeratosis, and parakeratosis forming solid or cystic areas, bearing some resemblance to the papillary subtype of human craniopharyngioma, which occurs almost exclusively in adults. An adamantinomatous epithelial component, i.e., peripheral palisading of cells, typical for most human craniopharyngiomas has never been observed in animals. Differential diagnoses in the rat included metastasizing squamous cell carcinoma from various sites, e.g., the oral cavity, salivary glands, esophagus, skin, lungs, preputial glands, and urinary bladder; carcinoma of Zymbal's gland; epidermoid cyst; aberrant craniopharyngeal structures; and teratoma. In this rat, there was no macroscopic and/or microscopic evidence of any other tumor. In view of possible difficulties in differentiating craniopharyngioma from metastasizing keratinizing squamous cell carcinoma, the classification of a multifocal lesion in the pituitary pars nervosa with widespread infiltration of neighboring structures and dissemination to the brain in a Sprague-Dawley rat as metastasizing craniopharyngioma does not appear convincing. Presence or absence of a compact tumor mass and keratohyaline granules within the neoplastic epithelium might aid in achieving the appropriate diagnosis. In the rat, epidermoid cysts have been described in the central nervous system but, in contrast to human beings, not in the pituitary region. These cysts may cause compression rather than invasion and exhibit a regular, occasionally hyperplastic stratified squamous epithelium with no atypia or dysplasia and regular layers of desquamated keratin. Aberrant craniopharyngeal structures are distinct, nonproliferative lesions within the rat pituitary pars nervosa that are composed of varying proportions of tubular or acinar glandular structures and cysts, consisting of flat, cuboidal, columnar, with occasional ciliation, or stratified squamous epithelium and, rarely, goblet cells, with no compression of adjacent tissues. The demonstration of tubular and glandular structures within a loose collection of fusiform cells in the pituitary pars nervosa of a Fischer rat or ciliation and goblet cells, producing large amounts of mucus, within a flat, cuboidal, or columnar epithelial lining, with no atypia, forming tubules and cysts in a pituitary lesion of a
Wistar rat\textsuperscript{10} did not support the diagnosis of a craniopharygioma\textsuperscript{6, 8}, but rather supported the diagnosis of aberrant craniopharyngeal structures\textsuperscript{14}. Pituitary teratoma is an extremely rare tumor-like lesion in a young rat and is composed of derivations from all three embryonic germ layers, i.e., neural tissue, cartilage, bone, striated muscle, adipose and connective tissue, squamous epithelium, and glandular structures\textsuperscript{23}, which were not present in the case described.

In conclusion, malignant craniopharyngioma should be considered a differential diagnosis in the rat when a tumor with stratified squamous epithelial features and locally aggressive growth pattern is revealed in the sellar or suprasellar region. The present case of a malignant craniopharyngioma extends further the spectrum of malignant pituitary tumors in the rat\textsuperscript{24, 25}.

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\textbf{Declaration of Conflicting Interests}

The authors declare that they have no potential conflicts of interests with respect to the research, authorship, and/or publication of this article.
References


Figure Legends

**Fig. 1.** Brain with a tumor in the sellar region (asterisk), indentation, and a solitary focus of invasion in the ventrolateral telencephalon (arrow). Note the distorsion of the median eminence (frontal section). H&E, x20.

**Fig. 2.** The tumor is composed of an irregular stratified squamous epithelium with marked hyperkeratosis and parakeratosis, forming solid areas or, predominantly, cysts of varying size filled with desquamated keratin, cellular debris, macrophages, and proteinaceous fluid. H&E, x160.

**Fig. 3.** Mitotic figures are fairly frequent (arrowheads) in the neoplastic stratified squamous epithelium with marked hyperkeratosis and parakeratosis on a delicate fibrovascular stroma. Cysts contain desquamated keratin, cellular debris, and macrophages. Inset: Irregular stratified squamous epithelium with no keratohyaline granules and cyst-like structures filled with desquamated keratin in somehow irregular layers or squames, H&E, x400.

**Fig. 4.** The tumor has deeply infiltrated the base of the skull, destroying most of the sphenoid bone. Tumor cells and desquamated keratin exhibit strong cytoplasmic immunoreactivity for pan-cytokeratin (Lu-5). PAP method, hematoxylin counterstain, Nomarski technique, x400.

**Fig. 5.** The tumor is directly abutting the pituitary gland. Minimal focal infiltration of the pituitary pars distalis is evident (arrows), which shows normal numbers and distributions of GH-immunoreactive cells. PAP method, hematoxylin counterstain, Nomarski technique, x400.

**Fig. 6.** Focus of tumor invasion associated with focal demyelination in the ventrolateral telencephalon from Fig. 1. Cords of neoplastic stratified squamous epithelium with no
keratohyaline granules and little or no hyperkeratosis and parakeratosis (arrows) are evident, with marginal hemorrhage and many macrophages, neutrophils and cellular debris within an adjacent cystic focus of demyelination (asterisk). H&E, x320.