Characteristics of Local Invasion of Spontaneous Pituitary Carcinoma in the F344 Rat

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Abstract: Spontaneous invasive pituitary carcinoma of F344 rats used in a 2-year carcinogenicity study were examined histopathologically and immunohistochemically. A modified trimming procedure was devised to increase the detection rate of invasive tumors. The pituitary was dissected together with the sphenoid bone and surrounding tissues, and sagittal or transverse sections were made. The incidence of invasive pituitary tumors was 8.4% for males and 14.8% for females under these preparation methods. The neoplastic cells invaded the capsule, sphenoid bone, surrounding veins, pars intermedia and pars nervosa, peripheral nerve, meninges, and brain. In most of the cases, neoplastic cells infiltrated into the posterior portion of the pituitary capsule. From these observations, the tumor was diagnosed as carcinoma. The incidence of carcinoma invasion into the sphenoid bone was higher in males than in females. Similarly, the proliferating cell nuclear antigen (PCNA) labeling index of carcinomas in males was significantly higher than in females. Almost all carcinomas showed positive cytoplasmic reaction for prolactin (PRL) by immunohistochemical examination. Extra-cranial metastases were not found in these carcinomas. The present study demonstrates that histopathological examination of the pituitary tumor section including the surrounding tissues is crucial for making an actual diagnosis of neoplasm, and that spontaneous pituitary carcinomas in the rat resemble invasive pituitary tumor in humans. (*J Toxicol Pathol* 1999; 12: 13~19)

Key words: Pituitary carcinoma, Pars distalis, Proliferating cell nuclear antigen (PCNA), prolactin (PRL), sphenoid bone, F344 rats.

Introduction

Pituitary tumor is a common neoplasm (in 2-year carcinogenicity studies) in aged rats of various strains^{1,2}. Most of these tumors are prolactin (PRL) secreting neoplasms arising from anterior acidophilic cells, and are thought to be benign. Thus, only a low incidence of malignant tumors is recognized^{1,3,4}. According to historical data of previous reports, the incidence of pituitary carcinoma in F344 rats has been reported to be 0-4.3% for males and 0-7.2% for females (**Table 1**⁵⁻¹⁴). In some cases of pituitary tumors, however, the authors have not distinguished benign and malignant tumors¹⁵⁻¹⁶. The majority of pituitary carcinomas were characterized by the infiltration of neoplastic cells into the dorsal portion of the brain^{1,3,17-19}. Neoplastic cells were also reported to invade the sphenoid bone with the ventral surrounding tissues rather than the brain¹⁴. The incidence of spontaneous pituitary carcinomas increases when the pituitaries are examined histopathologically together with surrounding tissues¹⁴. We detected invasive pituitary tumors in estrogen-treated rats by sagittal and transverse sections including surrounding tissues. The invasive neoplastic cells were noted in the capsule, surrounding veins, peripheral nerve, and sphenoid bone except for the brain²⁰. When the

pituitary is removed from the sphenoid bone for weighing, the opportunity to evaluate this invasion is lost. These data suggest that the detection of invasion in the pituitary tumor depends on the trimming site. Therefore, the dissection together with surrounding tissues including the sphenoid bone is considered to be an essential procedure to detect local invasion of pituitary tumors.

Despite the fact that numerous histological, immunohistochemical and ultrastructural studies have been done so far^{11,14,18,21-25}, the local invasion and cell proliferating activity of spontaneous pituitary tumors in rats remain to be elucidated. Since we observed several spontaneous pituitary tumors accompanied by local invasion in a rat 2-year carcinogenicity study, we evaluated these lesions by the sagittal or transverse sections to clarify their histopathological and immunohistochemical characteristics. In addition, the character of spontaneous pituitary carcinoma was compared with that of adenoma.

Materials and Methods

Animals

The F344/DuCrj rats (Charles River Japan Inc., Kanagawa) from control and treated groups utilized in a 2-year carcinogenicity study (167 males and 169 females) were selected for the present investigation. Among these rats, 39 rats consisting of 14 males and 25 females possessed invasive pituitary tumors in the pars distalis. Since the same severity, grade, and incidence of spontaneous neoplasia were observed in the control group as in the treatment group, they were not

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Table 1. Review of the Incidence of Spontaneous Pituitary Carcinoma Reported in F344 Rats

Age in Weeks	Number of animals		Pituitary adenoma (%)		Pituitary carcinoma (%)		Author (s)	Year	References
	Male	Female	Male	Female	Male	Female			
>110	1794	1754	11.3	29.2	0.1	1.1	Goodman <i>et al.</i>	1979	(5)
>107	459	459	14	34	0	1	Sher <i>et al.</i>	1982	(6)
>106	448	450	7	39	1	1			
>110	296	297	9.7	26.9	0.3	0	Maekawa <i>et al.</i>	1983	(7)
>110	2158	2262	21.7	44.0	2.4	3.5	Haseman <i>et al.</i>	1984	(8)
>110 ^{a)}	1614	1704	20.1	43.6	2.4	3.6	Haseman <i>et al.</i>	1985	(9)
>110 ^{b)}	1073	1062	23.0	36.1	2.1	3.0			
>110	960	959	12.4	28	0.4	0.7	Maita <i>et al.</i>	1987	(10)
>104	100	100	26	36	0	0	Sandusky <i>et al.</i>	1998	(11)
>109	469	354	16	45.2	1	2.5	Takai <i>et al.</i>	1989	(12)
>109	696	700	24.4	34.0	0.1	0.7	Iwata <i>et al.</i>	1989	(13)
>109 ^{c)}	139	138	25.9	42.8	4.3	7.2	Hosokawa <i>et al.</i>	1993	(14)

^{a)}: Untreated control, ^{b)}: Corn oil gavage control, ^{c)}: Examined transverse section of pituitary with sphenoid bone.

judged to be a compound-related change. The age of rats used in this study was 75–110 weeks for males, and 90–110 weeks for females. The animals were housed individually in a wire-mesh cage in an air-conditioned room (temperature, $23 \pm 2^\circ\text{C}$; relative humidity, $55 \pm 15\%$) with a 12-hr light/dark cycle. Basal diet (CRF-1, Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water were available *ad libitum*. The experimental protocol was prepared in accordance with the guidelines of the Institutional Animal Care and Use Committee of Daiichi Pharmaceutical Co., Ltd.

Histopathology

Moribund or dead animals found during the experimental periods and the animals killed at termination were subjected to a complete necropsy. Immediately after macroscopic examination, the pituitary was carefully removed together with other surrounding tissues including the capsule, surrounding veins, peripheral nerve, and sphenoid bone. These tissues were fixed in 10% neutral buffered formalin and then decalcified with buffered 25% formic acid for 10 days. The pituitary and surrounding tissues were sagittally²⁰ or transversely¹⁴ trimmed for detecting invasion into the surrounding tissues. Thirty-five other organs and gross lesions were also fixed in neutral buffered formalin. All specimens were embedded in paraffin wax, cut at $3\text{--}4\ \mu\text{m}$ in thickness and stained with hematoxylin and eosin (HE) via standard methods for light microscopic examination.

Pituitary lesions showing morphological features characterized by invasion of neoplastic cells into the surrounding organs/tissues were diagnosed as carcinoma^{14,17–20}

Immunohistochemistry

For immunohistochemical detection of proliferating cell nuclear antigen (PCNA) and prolactin (PRL), the pituitary sections were processed by a labeled streptavidin-biotin (LSAB) method²⁶. In brief, the paraffin section was deparaffinized and treated sequentially with mouse anti-PCNA antibody (1:500, DAKO Japan Co., Ltd., Tokyo) or rabbit anti-rat PRL antibody (1:1000, Chemicon International Co., Ltd., CA, USA) as the primary antibody. The sections were then stained with an LSAB kit (DAKO

Corporation Carpinteria, CA, USA) and counterstained with hematoxylin. Negative controls were performed by substituting phosphate buffered saline for the primary antibody.

Morphometric analysis

In PCNA immunohistochemistry, the section was scanned with a microscopic color image processor (SHR-6500, Keyence Co., Tokyo, Japan), and the positive nuclei per 100 pituitary pars distalis cells were represented as a proliferating cell index (%).

Statistical analysis

Quantitative data are expressed as the mean $\pm$ standard deviation (SD). Incidences and PCNA labeling indices were analyzed by Fisher exact test and Student's t-test or Aspin-Welch test. P-values less than 0.05 were considered significant.

Results

Gross examination and histopathology

The incidence of pituitary tumors was 31.1% (52/167) for males and 46.2% (78/169) for females, and that of invasive pituitary tumors (carcinomas) was 8.4% (14/167) for males and 14.8% (25/169) for females (**Table 2**). Macroscopically, all pituitary carcinomas were observed as enlarged pituitary masses (4–12 mm in diameter) having dark reddish coloration or being hemorrhagic in appearance. Some pituitary adenomas were normal in size and others had masses up to 12 mm in diameter. Histopathologically, pituitary adenomas and carcinomas were observed accompanied with blood-filled space consisting of desquamated endothelial cells in the sinus, hemorrhage and/or necrotizing foci. Cystic and solid pattern was prominent in various areas of the pituitary mass. The neoplastic cells were composed of small round to oval cells with chromophobic to acidophilic cytoplasm. The nuclei of neoplastic cells were larger than those of normal cells, and had atypical and irregular shape with multiple basophilic nucleoli. Pleomorphic cells with giant irregular nuclei (Fig. 1) were seen in 42.9% (6/14) of car-

Table 2. Incidence, Survival and Size of Spontaneous Pituitary Tumors in F344 Rats

	Pituitary Tumors	
	Adenoma	Carcinoma
Male^{a)}		
Pituitary tumor incidence (%) ^{b)}	38/167 (22.8)	14/167 (8.4)
Incidence of dead animals with pituitary tumor (%) ^{c)}	2/38 (5.3)	5/14 (35.7)
Week of first observation	75	93
Gross size of tumor mass	normal ^{d)} - ϕ 8 mm	ϕ 4-12 mm
Female^{a)}		
Pituitary tumor incidence (%) ^{b)}	53/169 (31.4)	25/169 (14.8)
Incidence of dead animals with pituitary tumor (%) ^{c)}	3/53 (5.7)	9/25 (36.0)
Week of first observation	90	95
Gross size of tumor mass	normal ^{d)} - ϕ 12 mm	ϕ 4-12 mm

^{a)}: Terminal-kill was performed on study week 105.

^{b)}: Number of tumor-bearing animals/number of animals examined microscopically.

^{c)}: Number of natural-death and moribund-kill animals with pituitary tumor/Number of lesion-bearing animals.

^{d)}: Not have any tumor mass.

ϕ : Diameter.

Fig. 1. Pituitary carcinoma in a male rat. Tumor cells show pleomorphism, and numerous giant bizarre nucleus (arrows). Sagittal section, H & E, $\times 700$.

Fig. 2. Invasive pituitary carcinoma in a female rat. Neoplastic cells invade into the posterior capsule (arrows). Sagittal section, H & E, $\times 75$.

cinomas for males and 16.0% (4/25) of carcinomas for females. Mitotic figures were frequently observed in the carcinoma containing pleomorphic cells. Often numerous neoplastic cells invaded the capsule, usually in the posterior portion as demonstrated by a sagittal section (Tables 3 and 4, Fig. 2). In addition, the neoplastic cells invaded the sphenoid bone including the periosteum and bone marrow cavity (Fig. 3a), surrounding veins (Fig. 3b), pars intermedia and pars nervosa (Fig. 3c), peripheral nerve (Fig. 3d), meninges (Fig. 3e), and brain (Fig. 3f). When the infiltration sites of neoplastic cells were examined, its incidence was 97.4%, 33.3%, 28.2%, 23.1%, 7.7%, 7.7%, and 2.6% in the capsule, surrounding vein, sphenoid bone, pars intermedia and pars nervosa, peripheral nerve, meninges, and brain, respectively (Table 3). Infiltration of carcinoma cells into the sphenoid bone was noted with extensive remodeling of bone (Fig. 3a) or through the foramen nutricium (Fig. 4). The incidence of infiltration of neoplastic cells into the sphenoid bone in males (64.3%) was significantly higher than that in females (16.0%). The carcinomas tended to invade posteriorly and laterally as demonstrated by sagittal and

transverse sections, respectively (Table 4).

PCNA labeling index

PCNA labeling index of adenomas and carcinomas in males was significantly higher than that in females (Table 5). Furthermore, the PCNA labeling index in the carcinomas was significantly increased compared to the adenomas. The pleomorphic carcinomas with giant nuclei displayed high levels in the PCNA labeling index (11.0%) mainly in males. In 6 carcinomas (3 males and 3 females), neoplastic cells replacing the sphenoid bone marrow cavity showed an elevated level in the PCNA labeling index (18.7%) at the invasion site. Although only one carcinoma (female rat) exhibited infiltration into the brain, its labeling index (1.5%) was similar or less to adenomas levels.

PRL immunohistochemistry

Pituitary carcinomas positively reacted to PRL antibody in 13/14 (92.2%) males and 25/25 (100%) females. PRL-positive carcinomas possessed secreting granules in the cytoplasm and its stain was paler in males than in females.

Fig. 3. Invasive pituitary carcinoma in female rats. a) Invasion into the sphenoid bone (arrows). Sagittal section, H & E, $\times 75$. b) Invasion into the surrounding vein (arrows). Transverse section of the brain, H & E, $\times 100$. c) Invasion into the pars intermedia and pars nervosa (arrows). I=pars intermedia (intermediate lobule), N=pars nervosa (posterior lobule). Sagittal section, H & E, $\times 150$. d) Invasion into the peripheral nerve (arrows). TN=Trigeminal nerve. Transverse section, H & E, $\times 75$. e) Invasion into the meninges (arrows). Transverse section of the brain, H & E, $\times 150$. f) Invasion into the brain parenchyma (arrows). Transverse section of the brain, H & E, $\times 150$.

However, the carcinoma cells in an area showing pleomorphic feature were considerably pale in both sexes.

Discussion

Pituitary carcinomas of the pars distalis were characterized by local invasion of neoplastic cells into the posterior capsule, sphenoid bone, and surrounding vein rather than the brain. Only neoplasms with local invasion were diagnosed as carcinoma, and no substantial cytological differences were found between adenomas and carcinomas. In our investigation, the incidence of spontaneous pituitary carcinomas was

8.4% for males and 14.8% for females, and these incidences were higher than those in previous studies (**Table 1**). Although it has been well-known that the occurrence of tumors is influenced by strain, breed, environment, diet, and diagnostic procedure^{1,9,15,16,27}, a difference in the incidence between previous data and ours may be due to a difference in a trimming site for histopathological examination. Hosokawa *et al.* (1993)¹⁴ reported that the incidence of pituitary carcinomas was increased (4.3% in male and 7.2% in female) when the sphenoid bone with surrounding tissues was examined by a transverse section. Their incidence was higher than others, though it was lower than ours. We

Table 3. Invasive Sites of Spontaneous Pituitary Carcinomas in F344 Rats

Sex	Number of animals ^{a)}	Invasion sites (%)						
		Capsule	Sphenoid bone ^{b)}	Surrounding vein	Another ^{c)} pars	Peripheral nerve	Meninges	Brain
Male	14	14 (100.0)	9 (64.3)**	3 (21.4)	2 (14.3)	2 (14.3)	0	0
Female	25	24 (92.0)	4 (16.0)	8 (32.0)	7 (28.0)	1 (4.0)	3 (12.0)	1 (4.0)
Total	39	38 (97.4)	13 (33.3)	11 (28.2)	9 (23.1)	3 (7.7)	3 (7.7)	1 (2.6)

^{a)}: Pituitary tumors in these animals were diagnosed as carcinomas.

^{b)}: The sphenoid bone included the periosteum and bone marrow cavity.

^{c)}: Pars intermedia and pars nervosa.

** $p < 0.01$ vs. incidence in females (Fisher exact test).

Table 4. Invasive Direction of Spontaneous Pituitary Carcinomas

Sex	Number of animals ^{a)}	Invasion directions (%)				
		Anterior	Posterior	Dorsal	Ventral	Lateral
Sagittal section plane						
Male	12	2 (16.7)	12 (100)	6 (50.0)	6 (50.0)	—
Female	15	5 (33.3)	12 (80.0)	3 (25.0)	11 (73.3)	—
Total	27	7 (25.9)	24 (88.8)	9 (33.3)	17 (63.0)	—

Transverse section plane						
Male	2	—	—	1 (50.0)	1 (50.0)	2 (100)
Female	10	—	—	1 (10.0)	3 (30.0)	5 (50.0)
Total	12	—	—	2 (16.7)	4 (33.3)	7 (58.3)

^{a)}: Pituitary tumors in these animals were diagnosed as carcinomas.

Fig. 4. Invasive pituitary carcinoma in a male rat. Tumor cells invade into the bone through the foramen nutricum (arrow). Sagittal section, H & E, $\times 1,000$.

examined the pituitary together with the surrounding tissues without dissecting them from the sphenoid bone to enhance the ability to detect cell infiltration. In our experience of the early stage of pituitary carcinoma, the neoplastic cells infiltrated the capsule, followed by expansive growth to the surrounding tissues. Meanwhile, in cases of infiltration into the anterior or dorsal portion, neoplastic cells passed through the Rathke's cleft, pars intermedia, and pars nervosa. In the posterior and lateral portions, however, neoplastic cells easily infiltrated the surrounding tissues because of no obstacle in the pituitary tissue. Taken together with the above information, it is suggested that the pituitary tumor specimens should

Table 5. Proliferating Cell Nuclear Antigen (PCNA) Labeling Indices for Pituitary Tumors

Sex	Pituitary Tumors	
	Adenoma	Carcinoma
Male	2.9 $\pm$ 2.9* N = 38	7.9 $\pm$ 4.4** N = 14
Female	1.7 $\pm$ 2.3 N = 53	4.5 $\pm$ 3.1 N = 25
Total	2.2 $\pm$ 2.6 N = 91	5.7 $\pm$ 3.9## N = 39

Values are means $\pm$ SD.

N: Number of animals examined.

* $p < 0.05$, ** $p < 0.01$ vs. index in females (Student's t -test).

$p < 0.01$ vs. index in adenoma (Aspin-Welch test).

be cut sagittally to detect the focal posterior and ventral invasion, and cut transversely to examine the lateral portion including the surrounding vein and intra-cranial peripheral nerve. Hence, evaluation of only the pituitary mass and brain is inadequate for accurate classification of pituitary adenomas and carcinomas in the rat.

Our findings that females showed a higher incidence in pituitary tumors than males is consistent with previously reported data (Table 1). The pathogenesis of the aging includes an increase in pituitary tumors and PRL secretion has been indeed shown by the reduction in secretory activity

of the tuberoinfundibular dopaminergic neurons of the hypothalamus²⁸, followed by hyperprolactinemia^{29,30}. On the other hand, age-related alterations in tyrosine hydroxylase phosphorylation and changes in the amount of tyrosine hydroxylase have been also involved in the impaired dopamine biosynthesis and increased incidence of pituitary tumors, especially in females^{1,28-31}. It has been reported that changes in the basal progesterone/estrogen ratio in aging animals play an important role in the induction of pituitary tumor^{23,31}. Thus, hypofunction of the hypothalamic neurons and hormone imbalance of the endocrine systems may cause pituitary tumors, at a higher incidence in females than in males.

PCNA labeling index, a marker of cell proliferation activity, was significantly higher in males than in females. This may be related to the high incidence of carcinoma cell invasion into the sphenoid bone and the cellular pleomorphism seen in males. Similarly, the pale staining in the PRL immunohistochemistry of pituitary carcinomas in males may mean an enhancement of malignancy along with immaturity, and a reduction in PRL production. PCNA labeling index of tumor cells was elevated in the invasion site such as sphenoid bone marrow compared to cells in the primary tumor mass. Since the development of pituitary tumors reduced the local blood flow by compression of the sinus and overlying veins, ischemia may occur in the primary tumor mass^{20,32}. As results, ischemia was considered to suppress the proliferation of carcinoma cells in the mass itself. The increase in the PCNA labeling index of invading tumor cells may be related to the rich blood flow in sphenoid bone marrow. On the other hand, colony stimulating factors (CSFs) have been reported to play a pivotal role in the development, differentiation and proliferation of the bone marrow cells³³. Therefore, it is likely that CSFs together with stable blood flow in the location were involved in the elevation in the PCNA labeling index of invading tumor cells. Although the majority of pituitary carcinomas in rats have been stated to infiltrate the brain^{1,3,17-19}, the one pituitary carcinoma that invaded the brain in the present investigation showed low levels of the PCNA labeling index.

There are few reports dealing with distant metastasis of pituitary carcinoma in both animals and humans^{4,34,35}. Extra-cranial metastasis was not observed in pituitary carcinomas despite invasion of tumor cells into the surrounding vein in the present study. In random autopsy cases in human beings, the incidence of pituitary tumor was reported to be 10-27%³⁶⁻³⁹. Histopathologically, cellular infiltration into the surrounding tissues was considered to be neoplasia in some cases^{34,35,40}, and its incidence was approximately 35-42%^{38,43}. These tumors were expressed in the term of 'invasive adenoma (invasive tumor)' which was referred to aggressive neoplasia among the non-metastatic pituitary tumors that were classified as benign tumor^{34,40,41}. Concerning the differential diagnosis between pituitary carcinoma and adenoma, there is still some controversy. The malignant pituitary tumors in rats was identified by the evidence of invasion into the surrounding tissue/organ^{14,17-20}. Furthermore, malignant tumors in this species were accompanied by aggressive cell behavior without metastasis. In contrast to rats, carcinoma in human beings was diagnosed only by the finding of metastasis or invasion into the brain^{34,40,41}. The above morphological characteristics in rats were almost comparable to the findings reported in humans^{34-36,39-41} with respect to invasion into the surrounding tissues and PRL

production without metastasis. It may be worthwhile to reclassify pituitary carcinomas in rats because the histopathological findings between humans and rats are similar.

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