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Concise Review

Histopathological and immunohistochemical features of proliferative lesions in the pituitary pars distalis of rats

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Abstract: Pituitary proliferative lesions derived from the endocrine cells of the pars distalis are frequently encountered and adenomas/carcinomas are a common cause of death in standard 2-year carcinogenicity studies using various rat strains, especially Sprague-Dawley. This report describes the immunohistochemical characteristics of pituitary tumors derived from the pars distalis in rats. Prolactin (PRL)-containing tumors are the most common, with PRL/growth hormone (GH) dual positive tumor masses (PRL/GH co-positive tumor masses) being more prevalent than only PRL-positive tumor masses (PRL single-positive tumor masses). GH-containing tumors are relatively numerous and many of these are also PRL/GH co-positive tumor masses. TSH-containing tumors are common in females. PRL-containing tumors have been shown to increase the incidence of hyperlactation in males and mammary adenomas/adenocarcinomas in females, suggesting that these masses are functional tumors. (DOI: 10.1293/tox.2020-0050; J Toxicol Pathol 2021; 34: 1–9)

Key words: pituitary, pars distalis, focal hyperplasia, adenoma, carcinoma, rat

Introduction

Proliferative lesions derived from the endocrine cells of the pituitary gland, especially those in the pars distalis, are commonly encountered as spontaneous background findings in carcinogenicity studies using various rat strains including Sprague-Dawley (SD), Fischer 344 (F344), and Wistar[1–15]. It is known that tumors of the pars distalis are a common cause of death in aged rats, especially females[2, 6]. In fact, a population required for statistical analysis is occasionally insufficient in carcinogenicity studies using rats because many of them are euthanized moribund or found dead due to pituitary tumors.

An entire population of aged rats may have one or multiple proliferative lesion(s) of the pars distalis, which include focal hyperplasias, adenomas, and carcinomas. Identification of the proliferative cell types is difficult using hematoxylin-eosin (HE)-stained specimens; therefore, immunohistochemical staining needs to be performed although an additional immunohistochemical characterization is usually not performed in standard 2-year carcinogenicity studies[1, 2]. It is widely accepted that rat tumors of the pars distalis consist mainly of prolactin (PRL)-producing cells and may occasionally also contain other hormone-producing cells including growth hormone (GH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), adrenocorticotropic hormone (ACTH), and luteinizing hormone (LH) sometimes in combination[1–4, 9–14, 16]. It is also known that two or more hormones may be produced from single tumor cells, e.g., PRL and GH[1, 2, 9, 14].

This concise review describes the histological features and incidence of focal hyperplasias, adenomas, and carcinomas of the pars distalis in SD, F344, and Wistar Hannover (WH) rats. Additionally, we investigated the proportion of hormone-producing cells in adenomas and carcinomas of the pars distalis in SD rats using immunohistochemical techniques in order to confirm and introduce the contents reported previously. We also investigated the relationship between PRL-containing pituitary tumors and mammary gland histological features since tumors derived from endocrine cells in the pars distalis may produce functional hormones accompanied by elevated plasma concentration[4, 11, 17].

Histological Features of Proliferative Lesions Derived from Endocrine Cells in Pars Distalis Identified Using HE-stained Specimens

Focal hyperplasia[1, 2, 4, 5, 9]

One or multiple foci composed of proliferative endocrine cells are present in the pars distalis (Fig. 2A and B). The proliferative cells are commonly chromophobic (Fig. 1A) or pale eosinophilic (Fig. 1B), and sometimes accompanied by hypertrophy with vacuolation (signet ring-like) (Fig. 1C).

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The number of endocrine granules varies and proliferative cells without granules are also common. The nuclear density of these lesions is higher than that of normal parenchyma. The proliferative foci consist of a single cell type, but other types of normal cells are occasionally scattered throughout the focus (Fig. 1A–D, arrows). Boundaries with surrounding parenchyma are not well demarcated, and there is little compression of the surrounding parenchymal tissues. Cellular pleomorphism or atypia is rare but can be observed occasionally (Fig. 1D, arrowheads). The diameter of these lesions is usually less than 50% of the width of the pars distalis and sinusoidal dilation (angiectasis) may be observed in some foci.

Adenoma[1, 2, 4, 5, 9, 12, 14]

One or multiple masses composed of proliferative endocrine cells are present in the pars distalis (Fig. 2C–F). The boundaries and compression within the surrounding parenchyma are more conspicuous than those of focal hyperplasias. There is no tumor cell invasion into the surrounding tissues. Although some large masses have some infiltrative growth into the pars intermedia and/or nervosa (Fig. 2E and F), the finding is not evidence for a diagnosis of carcinoma. As with focal hyperplasias, adenomas consist predominantly of chromophobic cells or cells with a pale eosinophilic cytoplasm. Adenomas consist of a single cell type, but cellular pleomorphism and atypia are more prominent than those in focal hyperplasias. The cells and the nuclei of these lesions are usually enlarged. Two or more masses may be observed in a single rat, although this is reasonably infrequent. In many cases, multiple proliferative lesions fuse together and appear as one large mass. Abundant blood sinusoids and blood cysts may also be present (Fig. 2F). The diameter of these lesions is usually larger than 50% of the width of the pars distalis.

Carcinoma[1, 2, 4, 5, 7]

Carcinomas are prominent large masses (Fig. 2G–I) that share a number of morphological features with adenomas including tumor cell nature and composition. In tumors of the pars distalis, malignancy is determined by tumor cell invasion into the surrounding tissues including the trigeminal nerve (Fig. 2G), blood vessels, sphenoid bone (Fig. 2H and I), and meninges and the brain (Fig. 2J). Preparation of a histopathological specimen of the pituitary mass attached to the sphenoid bone is necessary to confirm whether the tumor has invaded the surrounding tissue. Distant metastases of these tumor cells are extremely rare.

Growth pattern of proliferative lesions

The growth pattern of proliferative lesions from the pars distalis is shown in Fig. 2. All the figures of the lesions

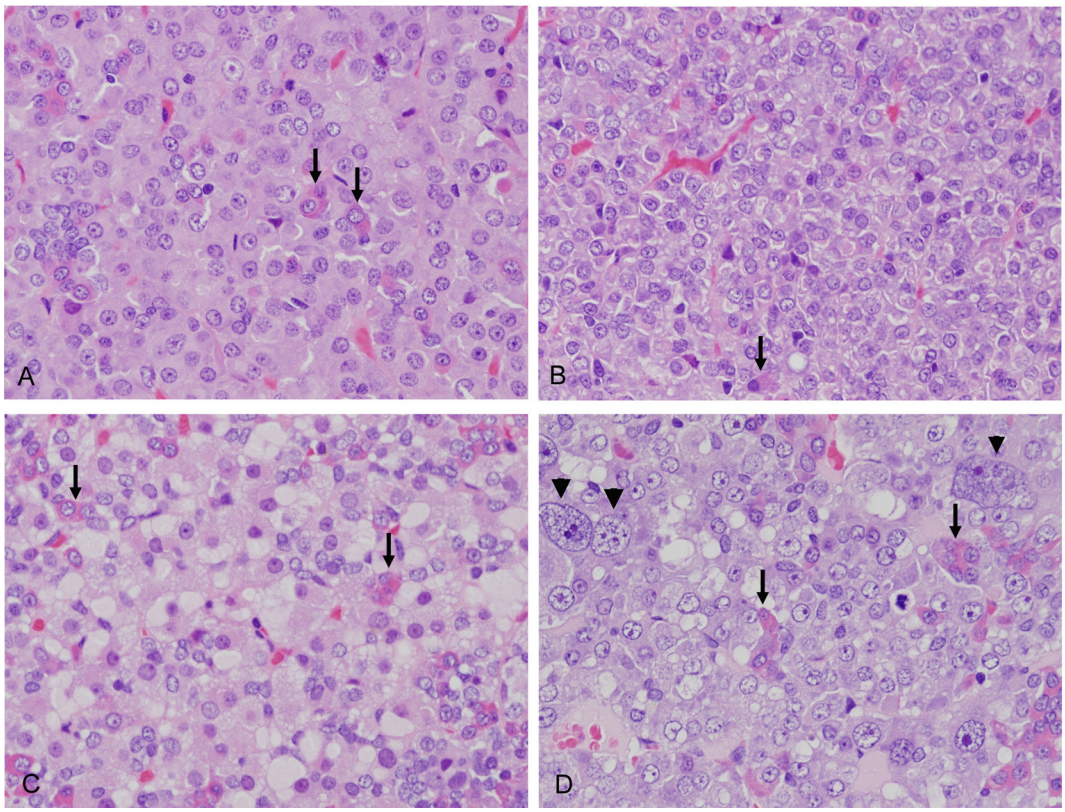

Fig. 1. Morphological features of the pituitary proliferative lesions in the pars distalis. A: Focal hyperplasia consisting of chromophobic cells. B: Focal hyperplasia consisting of pale eosinophilic cells. C: Focal hyperplasia consisting of pale basophilic cells with cytoplasmic vacuolation. D: Focal hyperplasia with cellular pleomorphism or atypia (arrowheads). Arrows: Scattered normal acidophils.

were taken at the same magnification, except for Fig. 2J (brain). Focal hyperplasias (Fig. 2A and B) can develop into small adenomas compressing the surrounding parenchyma (Fig. 2C). Small adenomas can grow further and develop into large adenomas compressing the pars intermedia and nervosa (Fig. 2D–F), and then into adenomas large enough to occupy the whole pituitary gland (Fig. 2E and F). In the continuous expanding growth, adenomas can ultimately become carcinomas that can invade the surrounding tissues including the trigeminal nerve (Fig. 2G, arrow), sphenoid bone (Fig. 2H and I, arrows), and/or meninges and the brain (Fig. 2J). Other endocrine tumors, like the C-cell tumors of the thyroid gland, pheochromocytomas of the adrenal gland, or islet cell tumors of the pancreas, demonstrate similar growth patterns. To date, we have never experienced instances where carcinomas from the pars distalis have caused distant metastasis. Adenomas, especially large ones, have the potential to be fatal, even if they never evolve into carcinomas; this is because their continuous expanding growth may lead to compression of the brain, which can result in death. Unlike in humans and companion animals, diagnosis in laboratory animals does not evaluate prognosis because these animals are necropsied at specified points defined by the examination protocol or found dead and euthanized moribund. However, a diagnosis of focal hyperplasia, adenoma or carcinoma is required at the time of necropsy regardless of prognosis and needs to reflect any incidence of proliferative lesions in any carcinogenicity studies of rodents.

Interpretation of cellular pleomorphism and atypia

As a general interpretation, cellular pleomorphism and atypia are important findings when considering if a tumor has malignant potential. However, in rodent endocrine gland tumors, including pituitary tumors, these findings do not necessarily coincide with malignancy[2]. Therefore, a diagnosis of "carcinoma *in situ*" is not applied to small proliferative foci containing atypical cells without boundary or compression of the surrounding parenchyma, and the foci should be diagnosed as focal hyperplasia (Fig. 1D). In our experience, atypical cells are more frequently seen in the proliferative lesions of WH rats, even in focal hyperplasias and small adenomas. On the other hand, the incidence of carcinomas is not different between WH rats and the other strains. This supports the interpretation that cellular pleomorphism or atypia does not always correlate with malignant transformation in rat pituitary tumors. However, it is also true that cellular pleomorphism and atypia are more frequently seen in tumors, especially in large adenomas and carcinomas.

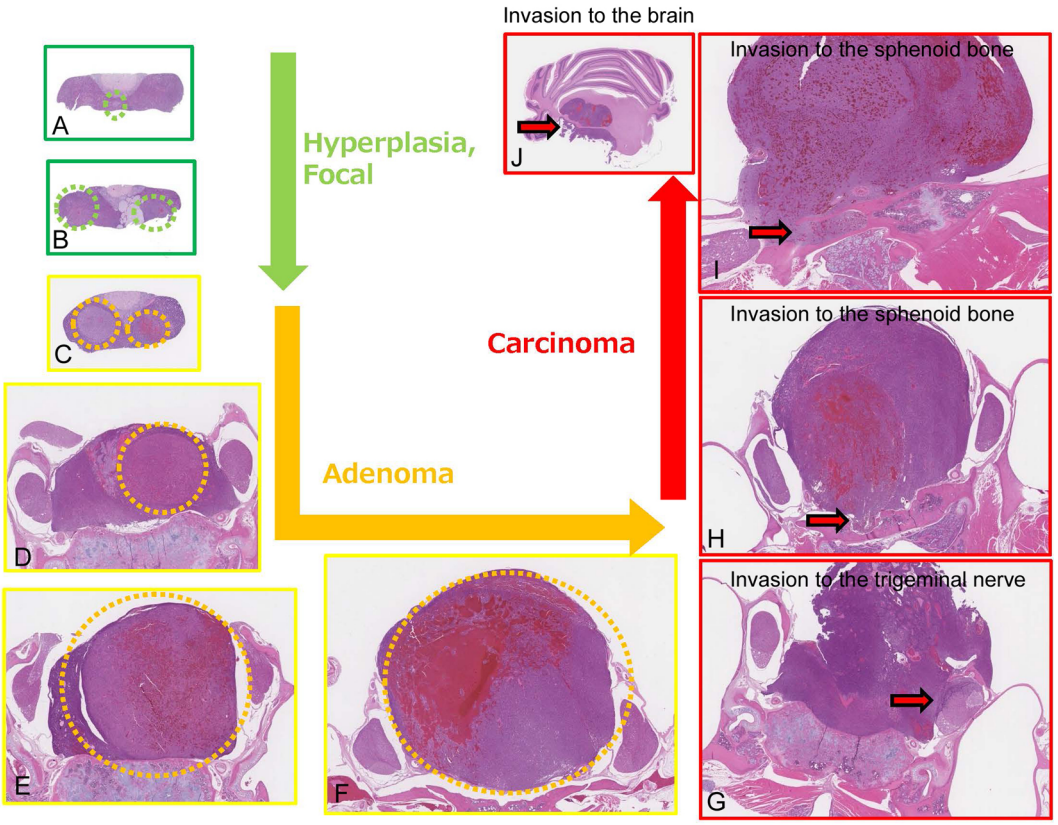

Fig. 2. Development of pituitary proliferative lesions in the pars distalis. All figures are taken at the same magnification except for 2J (brain). Figures with green borders show focal hyperplasia, those with yellow borders show adenomas and those with red borders show carcinoma. Invasion is shown by red arrows.

Incidence of Pituitary Proliferative Lesions in the Pars Distalis

The incidence of focal hyperplasias, adenomas and carcinomas in the pars distalis was investigated using F344 rats (220 males, 170 females), SD rats (469 males, 470 females), and WH rats (100 males, 100 females) used in 2-year carcinogenicity background studies. The results of this analysis are summarized in Fig. 3. In all three strains, the incidence of focal hyperplasias was higher in males than that in females, while the incidence of adenomas and carcinomas was higher in females than that in males. The reasons for this inverse correlation are thought to be due to (1) tumors (large masses) occupying the whole pituitary parenchyma which would mask focal hyperplasias (small foci) and (2) focal hyperplasias more quickly developing into tumors in females compared to males. The incidence of adenomas and carcinomas was highest in female SD rats.

Immunohistochemical Analysis

We further analyzed the positive areas within the adenomas and carcinomas using antibodies against hormones produced by the pituitary gland. We analyzed 26 masses from 24 male and 54 masses from 36 female SD rats, where some of the animals presented multiple tumor masses. The procedures and primary antibodies used in this immunohistochemical analysis are shown in Table 1. Paraffin-embedded sections were stained for prolactin (PRL), growth hormone (GH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), and adrenocorticotropic hormone (ACTH)[18]. The polymer kit (HISTOFINE Simple Stain Rat MAX-PO (MULTI), Nichirei Bioscience Inc., Tokyo, Japan) was used for secondary antibody. Features of these adenomas and carcinomas are shown in Fig. 4. Generally, the tumor cells that stained positively for PRL (PRL-positive tumor cells) were small to medium in size and presented with and without pale eosinophilic cytoplasmic granules in the HE-staining. PRL-positive staining appeared at the perinuclear area (Fig. 4A) or in the whole cytoplasm (Fig. 4B). GH-positive tumor cells were small to medium-sized cells without distinct granules in the HE staining, and GH-positive staining usually appeared in the whole cytoplasm (Fig. 4C). TSH-positive tumor cells tended to be larger and more angular with a vesicular nucleus and a prominent nucleolus in the HE-staining, and TSH-positive staining usually appeared weakly but sometimes strongly throughout the cytoplasm (Fig. 4D). Strongly TSH-positive tumor cells were also positive for FSH (TSH-FSH double-positive tumor cells) (Fig. 4E). Besides these, some tumor cells reacted positively for two hormones. In our investigation, the most common type of double positive tumor cells was PRL-GH double-positive tumor cells (Fig. 4F), with other types such as PRL-TSH and GH-TSH double-positive tumor cells. Tumor cells that were immunohistochemically negative for all the hormones (all negative tumor cells) were also observed. Some tumor masses consisted of two or more immunohistochemical types of tumor cells. For example, PRL and GH dual positive tumor masses (PRL/GH co-positive tumor mass) consisted of at least two types of cells from PRL single-positive, GH single-positive, and PRL-GH double-positive tumor cells and sometimes included all negative tumor cells. In females, co-positive tumor masses were more frequent in large adenomas and carcinomas. An example of an adenoma containing two proliferative lesions is shown in Fig. 5. One lesion was PRL/GH co-positive and consisted of small proliferative cells with and without pale eosinophilic granules; the other was TSH/FSH co-positive and consisted of large angular cells with a vesicular nucleus

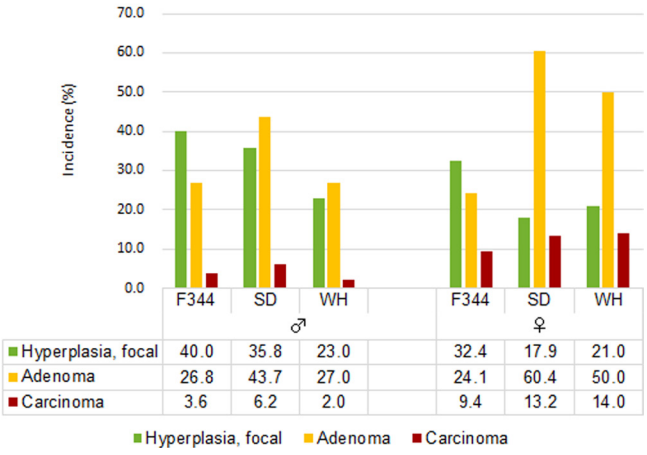

Fig. 3. Incidence of pituitary proliferative lesions in the pars distalis of SD, F344, and WH rats in 2-year carcinogenicity studies. The incidence of focal hyperplasias is higher in males than that in females. Inversely, the incidence of adenomas and carcinomas is higher in females than that in males and is the highest in female SD rats.

Table 1. Procedure and Primary Antiboides Used in Immunohistochemical Analysis

Antibody	Supplier	Host	Clone	Dilution	Antigen retrieval
Prolactin (PRL)	abcam	Rabbit	Monoclonal	×8,000	None
Growth hormone (GH)	BIO-RAD Laboratories	Rabbit	Polyclonal	×8,000	None
Thyroid stimulating hormone (TSH)	Millipore	Rabbit	Polyclonal	×5,000	None
Follicle stimulating hormone (FSH)	Millipore	Rabbit	Polyclonal	×5,000	None
Adrenocorticotropic hormone (ACTH)	Santa Cruz Biotechnology	Mouse	Monoclonal	×1,000	Antigen retrieval solution (pH 6.0), 62–65°C, Overnight

and a prominent nucleolus. This adenoma is thought to be the result of fusions between two or more proliferative lesions.

We investigated the proportion of cells that were positive for each hormone in each tumor mass. This analysis was classified into five scores based on the visual ratio of the positive cells in the whole tumor area. These scores were as follows: score 0, 0% to 5%; score 1, 5% to 25%; score 2, 25% to 50%; score 3, 50% to 75%; score 4, 75% and above. Graphical summaries of these scores are shown in

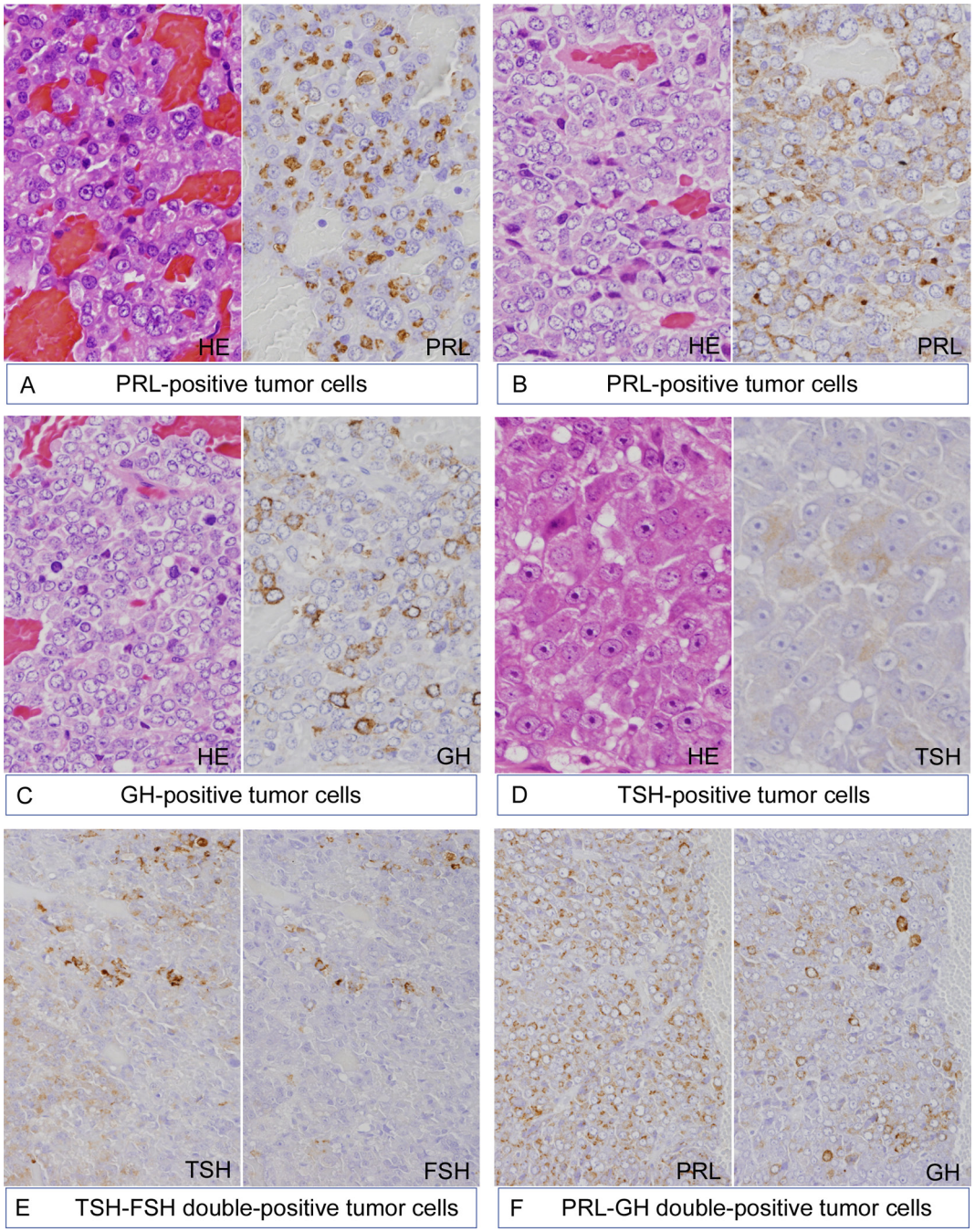

Fig. 4. Immunohistochemical features of the tumor cell types in adenomas of the pars distalis. A (PRL-positive tumor cells): Small to medium-sized tumor cells with pale eosinophilic granules are stained for PRL at the perinuclear area. B: (PRL-positive tumor cells): Small to medium-sized tumor cells without distinct granules are stained for PRL in the whole cytoplasm. C (GH-positive tumor cells): Small to medium-sized tumor cells without distinct granule are stained for GH in the whole cytoplasm. D (TSH-positive tumor cells): Large angular tumor cells with a vesicular nucleus and a prominent nucleolus are usually stained weakly for TSH in the whole cytoplasm, and some tumor cells are stained strongly for TSH. E (TSH-FSH double-positive tumor cells): TSH-positive tumor cells stained strongly are also positive for FSH. F (PRL-GH double-positive tumor cells): Some PRL-positive tumor cells are also positive for GH.

Fig. 6. PRL reacted positively in 20/26 tumor masses (77%) of males and 28/54 tumor masses (52%) of females, suggesting that PRL-producing cells are predominant in pituitary tumors of the pars distalis. PRL/GH co-positive tumor masses noted in 13/26 (50%) of males and 20/54 (37%) of females were more frequent than PRL single-positive tumor masses noted in 6/26 (23%) of males and 6/54 (11%) of females. However, the proportion of PRL-positive cells was higher than that of GH-positive cells in most PRL/GH co-positive tumor masses. In males, tumor masses could be classified as PRL single-positive, PRL/GH co-positive, PRL/TSH co-positive or PRL/GH/TSH co-positive masses; except for "null-cell adenomas" which consisted only of all negative tumor cells. Besides those, in females, tumor masses were classified as GH single-positive, TSH single-positive, GH/TSH co-positive, TSH/FSH co-positive, or GH/TSH/FSH co-positive masses, suggesting that the variation of producing hormone in tumor cells was wider in females than in males. Interestingly, TSH-containing tumors may be relatively common in females as they were noted in 18/54 (33%) masses of females, and 8 female tumors reacted positively for only TSH. Most TSH-containing tumors were located close to the sphenoid bone (Fig. 5). 6/26 (23%) masses of males and 6/54 (11%) masses of females were "null-cell adenomas", and almost all of these (6 masses in males and 5 masses in females) were large masses.

In previous reports published in the 1970s and 1980s, the incidence of PRL single-positive tumors was 50% to 70% and the incidence of PRL/GH co-positive tumors was low in rat pituitary tumors[9–13]. McComb *et al*. reported that PRL single-positive adenomas were the most common, accounting for 47.2%, and PRL/GH co-positive adenomas were less common, accounting for 10.9% of their SD rat samples[14]. Our results suggested that PRL/GH co-positive tumors were the most common. Additionally, previous reports did not describe the prevalence of TSH-containing tumors in females. In the 30 years since then, the difference in the immunohistochemical results may be attributed to the development of antibodies and technologies for activation of antigens for immunostaining methods.

Pituitary-specific transcription factor 1 (Pit-1) is a prototypic member of the Lit-Oct-Unc transcription factor family and is known to be involved in the functional differentiation, retention, and proliferation of GH-, PRL-, and TSH-producing cells[19, 20]. This suggests that pituitary tumors from the pars distalis in rats are predominantly composed of the tumor cells showing functional differentiation related to transcriptional factor of Pit-1.

Relationship Between PRL-containing Pituitary Tumors and Incidence of Mammary Lesions

It is known that there is a positive correlation between plasma PRL concentrations and the intensity of immunohistochemical staining for PRL in rat pituitary tumors[17]. We investigated the relationship between PRL-containing tumors and mammary tumors using 36 female SD rats with pituitary tumors (Fig. 7). Immunohistochemical staining for

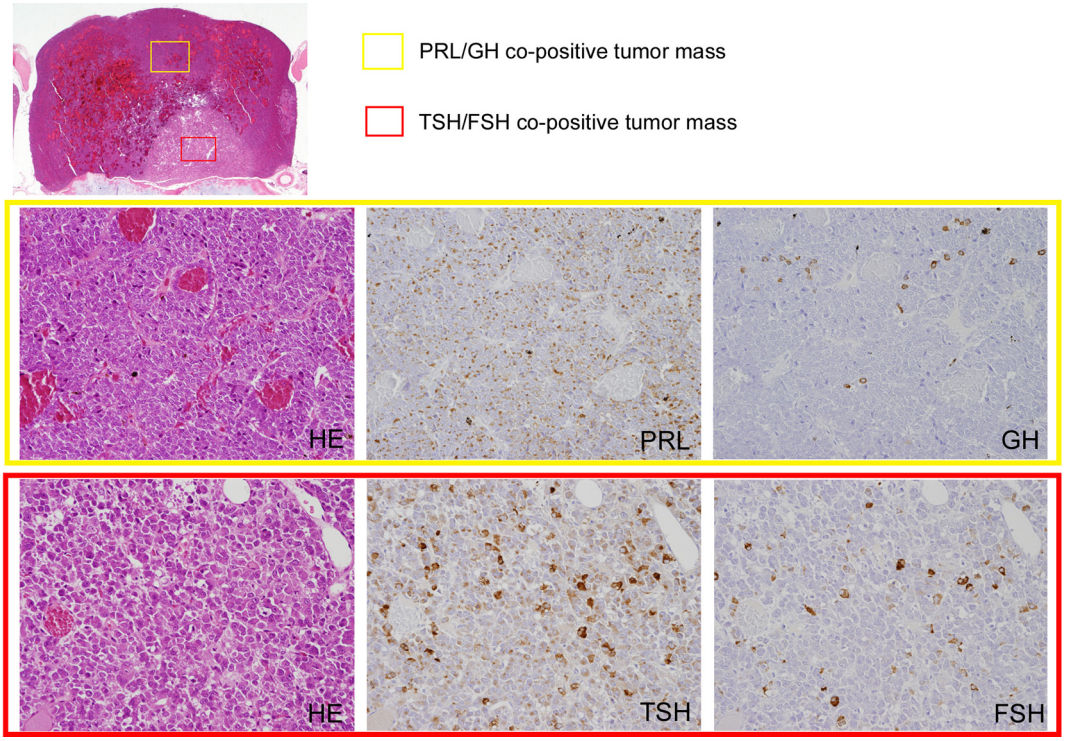

Fig. 5. Histological features of adenoma containing two proliferative lesions of different cell types. The area within the yellow circle is PRL/GH co-positive tumor mass, and the area within the red circle is TSH/FSH co-positive tumor mass.

Fig. 6. Immunohistochemical proportion of pituitary tumors from the pars distalis. Adenomas retaining the pituitary morphology are classified as small, and adenomas which do not retain the pituitary morphology are classified as large. The scores for the ratio of the positive cells in the whole tumor areas are as follows: score 0, 0% to 5%; score 1, 5% to 25%; score 2, 25% to 50%; score 3, 50% to 75%; score 4, 75% and above.

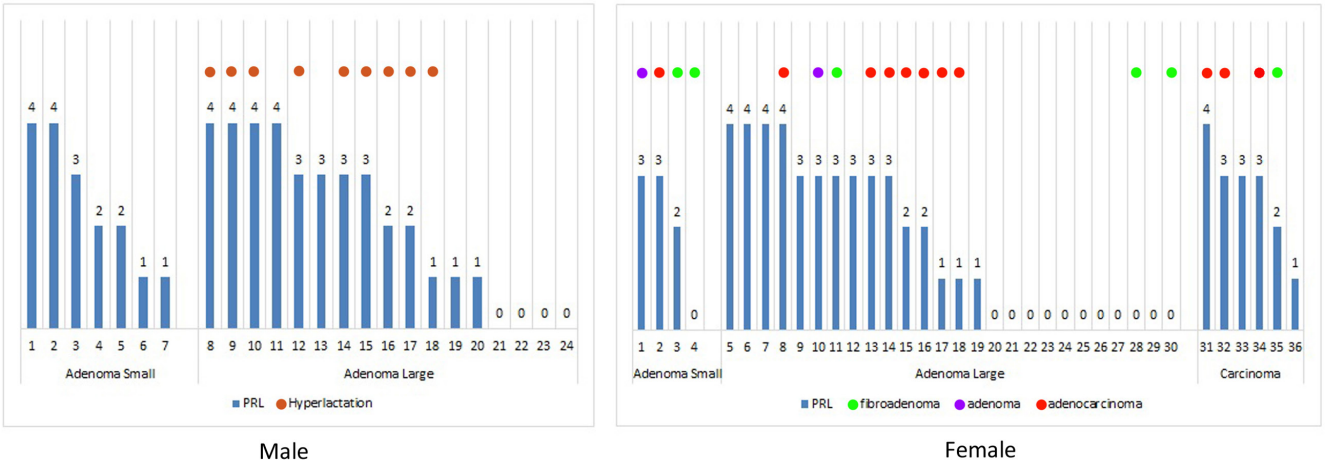

Fig. 7. Relationship between PRL-containing pituitary tumors and incidence of mammary lesions. The scores for the ratio of PRL-positive cells in the whole tumor area are as follows: score 0, 0% to 5%; score 1, 5% to 25%; score 2, 25% to 50%; score 3, 50% to 75%; score 4, 75% and above. PRL-containing tumors have a relationship with the incidence of hyperlactation in males and adenomas/adenocarcinomas in the mammary gland.

PRL was classified into five scores: score 0, 0% to 5%; score 1, 5% to 25%; score 2, 25% to 50%; score 3, 50% to 75%; score 4, 75% and above, similar to the scoring for the immunohistochemical analysis. Mammary tumors were classified as fibroadenomas, adenomas, or adenocarcinomas. Mammary tumors from rats with non-PRL-containing pituitary tumors were only fibroadenomas, while those from rats with PRL-containing pituitary tumors included mammary adenomas and adenocarcinomas as well as fibroadenomas. The incidence and malignancy of these mammary tumors did not correlate with PRL-positive score. Generally, mammary tumors can occur in SD female rats without pituitary tumors. Additionally, it is known that PRL cells and PRL content in the pituitary increase with aging in female rats[21]. In other words, non-PRL-containing pituitary tumors may lower the risk for mammary tumors with prominent glandular proliferation, especially adenomas and adenocarcinomas because PRL-negative tumor cells occupy the pituitary parenchyma, which leads to reduced plasma PRL concentrations. Conversely, the occurrence of mammary adenomas and adenocarcinomas maintained in female rats with PRL-containing tumors suggests that PRL-containing tumors are functional. In males, we investigated the relationship between PRL-containing tumors and hyperlactation of the mammary glands showing ductal dilation using 24 SD rats with pituitary tumors since there was low incidence of mammary tumors (Fig. 7). Hyperlactation was found in rats with large PRL-containing adenomas but was not found in rats with normal pituitary, non-PRL-containing adenomas, or small PRL-containing adenomas. Additionally, the incidence of hyperlactation had a tendency to increase depending on the PRL-positive scores. These findings suggest that hyperlactation in male rats with large PRL-containing tumors reflect the elevation of plasma PRL concentration.

Conclusion

PRL-containing tumors are the most common in pituitary tumors from the pars distalis, and PRL/GH co-positive tumor masses are more frequent than PRL single-positive tumor masses. PRL-containing tumors can be considered as functional tumors in both males and females due to their effect on mammary gland lesions. GH-containing tumors are relatively numerous and many of these do not consist of GH-positive tumor cells only but mixed with PRL-positive tumor cells. It remains unclear whether GH-containing tumors are functional or not. TSH-containing tumors occur occasionally in females and are rare in males, and no hyperplastic or neoplastic lesions derived from thyroid follicular cells were detected in rats with TSH-containing pituitary tumors in our investigation. Functional tumors in the pituitary of dogs are most likely derived from corticotroph (ACTH-secreting) cells in either the pars distalis or the pars intermedia[3, 4] and may induce a clinical syndrome of cortisol excess (Cushing's disease)[4]. In our SD rat investigation, ACTH-containing tumors were not detected, though a small number of tumors containing immunohistochemically ACTH-positive cells were described in previous reports[3, 4, 9, 13, 14].

Disclosure of Potential Conflicts of Interest: The authors have no conflicts of interest to declare.

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References

1. Brändli-Baiocco A, Balme E, Bruder M, Chandra S, Hellmann J, Hoenerhoff MJ, Kambara T, Landes C, Lenz B, Mense M, Rittinghausen S, Satoh H, Schorsch F, Seeliger F, Tanaka T, Tsuchitani M, Wojcinski Z, and Rosol TJ. Nonproliferative and proliferative lesions of the rat and mouse endocrine system. J Toxicol Pathol. **31**(Suppl): 1S–95S. 2018. [Medline] [CrossRef]
2. Remick AK, and Brown DL. Endocrine system. Pituitary gland. Hyperplastic and neoplastic lesions. Pars distalis. In: Boorman's Pathology of the Rat, 2nd ed. Suttie AW (ed). Academic Press, Amsterdam. 638–643. 2018.
3. Greaves P. Endocrine glands. Pituitary gland. Pituitary neoplasia. In: Histopathology of Preclinical Toxicity Studies, 4th ed. Greaves P (ed). Academic Press, Amsterdam. 736–740. 2012.
4. Wallig MA. Endocrine gland. Pituitary gland. In: Fundamentals of Toxicologic Pathology, 3rd ed. Wallig MA, Haschek WM, Rousseaux CG, Bolon B, and Mahler BW (eds). Academic press, London. 586–591. 2018.
5. Majka JA, Solleveld HA, Barthel CH, and Zwieten MJV. Proliferative lesions of the pituitary in rats. In: Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington, DC. 1–8. 1990.
6. Son WC. Factors contributory to death of young Sprague-Dawley rats in carcinogenicity studies. Toxicol Lett. **153**: 213–219. 2004. [Medline] [CrossRef]
7. Hosokawa S, Fukuta T, Imai T, Sonoda J, Aoki T, Hayakawa K, Motooka S, and Nakanowatari J. Pituitary carcinoma of pars distalis as a common neoplasm in Fischer-344 rats. Toxicol Pathol. **21**: 283–287. 1993. [Medline] [CrossRef]
8. Suzuki H, Mohr U, and Kimmerle G. Spontaneous endocrine tumors in Sprague-Dawley rats. J Cancer Res Clin Oncol. **95**: 187–196. 1979. [Medline] [CrossRef]
9. Sandusky GE, Van Pelt CS, Todd GC, and Wightman K. An immunocytochemical study of pituitary adenomas and focal hyperplasia in old Sprague-Dawley and Fischer 344 rats. Toxicol Pathol. **16**: 376–380. 1988. [Medline] [CrossRef]
10. Barsoum NJ, Moore JD, Gough AW, Sturgess JM, and De La Iglesia FA. Morphofunctional investigations on spontaneous pituitary tumors in Wistar rats. Toxicol Pathol. **13**: 200–208. 1985. [Medline] [CrossRef]
11. Trouillas J, Girod C, Claustrat B, Curé M, and Dubois MP. Spontaneous pituitary tumors in the Wistar/Furth/Ico rat strain. An animal model of human prolactin adenoma. Am J Pathol. **109**: 57–70. 1982. [Medline]
12. Kovacs K, Horvath E, Ilse RG, Ezrin C, and Ilse D. Spontaneous pituitary adenomas in aging rats. A light microscopic, immunocytological and fine structural study. Beitr Pathol. **161**: 1–16. 1977. [Medline] [CrossRef]
13. Nagatani M, Miura K, Tsuchitani M, and Narama I. Relationship between cellular morphology and immunocytological findings of spontaneous pituitary tumours in the aged rat. J Comp Pathol. **97**: 11–20. 1987. [Medline] [CrossRef]
14. McComb DJ, Kovacs K, Beri J, and Zak F. Pituitary adenomas in old Sprague-Dawley rats: a histologic, ultrastructural, and immunocytochemical study. J Natl Cancer Inst. **73**: 1143–1166. 1984. [Medline]
15. Hojo M, Sakamoto Y, Maeno A, Tayama K, Tada Y, Yuzawa K, Ando H, Kubo Y, Nagasawa A, Tanaka K, Yano N, Kaihoko F, Hasegawa Y, Suzuki T, Inomata A, Moriyasu T, Miyajima K, and Nakae D. A histopathological analysis of spontaneous neoplastic and non-neoplastic lesions in aged male RccHan:WIST rats. J Toxicol Pathol. **33**: 47–55. 2020. [Medline] [CrossRef]
16. Berry PH. Effect of diet or reproductive status on the histology of spontaneous pituitary tumors in female Wistar rats. Vet Pathol. **23**: 610–618. 1986. [Medline] [CrossRef]
17. van Nesselrooij JHJ, Kuper CF, and Bosland MC. Correlations between presence of spontaneous lesions of the pituitary (adenohypophysis) and plasma prolactin concentration in aged Wistar rats. Vet Pathol. **29**: 288–300. 1992. [Medline] [CrossRef]
18. Furukawa S, Nagaike M, and Ozaki K. Databases for technical aspects of immunohistochemistry. J Toxicol Pathol. **30**: 79–107. 2017. [Medline] [CrossRef]
19. Yasuno H, Watanabe T, Miyamoto Y, Kandori H, Yamasaki H, and Fukuda R. Expression of steroidogenic factor 1 and pituitary specific transcription factor 1 in rat pituitary adenomas. J Toxicol Pathol. **26**: 209–213. 2013. [Medline] [CrossRef]
20. Sano N, Umeoka K, Ooyama K, Tahara S, Takumi I, Teramoto A, and Osamura Y. [Pituitary tumors from molecular-biological aspects]. J Nippon Med Sch. **68**: 69–73. 2001. (in Japanese). [Medline] [CrossRef]
21. Oishi Y, and Satoh H. Endocrine system. Pituitary gland. In: Toxicologic Histopathology. Japanese Society of Toxicologic Pathology (ed). Nishimura Shoten, Tokyo. 477–493. 2018 (in Japanese).