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 Wistar1–15. It is known that tumors of the pars distalis are a common cause of death in aged rats, especially females2, 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 studies1–2. 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 combination1–4, 9–14, 16. It is also known that two or more hormones may be produced from single tumor cells, e.g., PRL and GH1, 2, 9, 14.

This concise review describes the histological features and incidence of focal hyperplasias, 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 concentration4, 11, 17.

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

Focal hyperplasia1, 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).
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**

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**

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.](image-url)
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. 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). 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 (FSH-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](image-url) 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 Antibodies 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 le-
sions.

We investigated the proportion of cells that were posi-
tive 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. 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.
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. 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 and may induce a clinical syndrome of cortisol excess (Cushing’s disease). 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.

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

**Acknowledgments:** We would like to thank Ms. Miwako Ishii and Mr. Takayoshi Ito for their excellent technical assistance and Mr. James Kenji Harada and Ms. Kanae Tamasukuri for their assistance in language refinement of this manuscript.
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