Spontaneous Prolactin Transplantable Tumor in the Wistar/Furth Rat (SMtTW): A New Animal Model of Human Prolactinoma

Jacqueline Trouillas, Christian Girod, Bruno Claustrat, Marie-Odile Joly-Pharaboz, and Pascale Chevallier

Laboratoire d’Histologie, Faculté de Médecine Alexis Carrel, Lyon, France [J. T., C. G., P. C.]; the Service de Radiopharmacie et de Radioanalyse, Hôpital Neurologique, Lyon, France [B. C.]; and the Laboratoire de Biochimie, Hôpital Edouard Herriot, Lyon, France [M-O. J.-P. J.]

ABSTRACT

Two spontaneous prolactinomas, removed from 28-mo-old female Wistar/Furth rats, were grafted by serial passages under the kidney capsule and the skin in 117 females of the same consanguineous strain. The hosts, aged between 2 and 10 mo, were free of estrogen treatment. These transplantable tumors, named SMtTW and SMtTW₂, were studied until the fifth serial passage. The percentage of success was 100% under the kidney capsule and 20% under the skin. From the radioimmunoassays of prolactin (PRL), growth hormone, and adrenocorticotropic hormone and the immunocytochemical results, the tumors secrete PRL only. The PRL tumoral secretion was detected after 3 to 5 mo of graft; at 8 mo, mean plasma PRL values reached 5150 ng/ml (normal value, 15.2 ng/ml). Plasma growth hormone and adrenocorticotropic hormone values remained normal. Like the primary tumors, the grafted tumors were benign, grew slowly, and were sparsely granulated well-differentiated prolactinomas with exocytosis. They remained identical during the first serial passages. The secretion and the growth of SMtTW₂ were inhibited by bromocriptine.

In the light of our knowledge of the human prolactinoma, the spontaneous transplantable prolactinoma of the rat may be considered to be an animal model closer to the human pathology than the estrogen-induced “tumors” and the induced transplantable tumors. It is easier to use than the spontaneous prolactinoma of the rat.

INTRODUCTION

Great progress in the understanding of human pituitary tumors has been made, but the pathogenesis and the factors of their growth are not well known. To study the factors of pituitary carcinogenesis, researchers have used animal models. The most studied experimental tumors are estrogen-induced “tumors” (1–6). However, some recent studies argue against the opinion that estrogens alone induce pituitary tumors (Refs. 4 and 5; Footnote 3). The pituitaries of estrogen-treated rats were transplanted in estrogen-treated hosts only. After some passages, they were able to grow in animals without estrogen treatment. Head irradiation and carcinogens also induce transplantable pituitary tumors (6). These induced transplantable tumors (MtTF₄, MtTF₅, MtTF₁₀, MtTF₁₅, 7315a), which were widely studied (2, 7–11), differ from most human pituitary tumors by their malignancy, their undifferentiated aspects, and their multisecretion. Thus, although they are interesting, they are not identical to human pathology.

More recently, spontaneous pituitary tumors in old rats (12–17) and dogs (18) were described. The comparison of some of these spontaneous animal tumors with human pituitary adenomas led us to consider them as good models of the human prolactinoma. We previously showed that the spontaneous prolactinomas of female Wistar/Furth rats (WF/Ico) are very similar to human prolactinoma (17). This model is, however, difficult to use because it takes a long time to obtain enough suitable tumor-bearing animals, thereby making it a costly procedure. Moreover, the tumor is small and awkwardly located. To avoid these experimental difficulties, we grafted spontaneous prolactinomas under the kidney capsule and the skin of consanguineous animals of the same strain. These unselected prolactinomas were directly transplantable without treatment of the hosts. The grafted tumors remained identical during the first serial passages studied. It is the first time that transplantable tumors from spontaneous and benign pituitary tumors were described.

We present here the graft procedure and the characteristics and advantages of this new model: the spontaneous transplantable prolactinoma in an inbred rat strain (WF/Ico). In memory of Jacob Furth, the pioneer in this field, and to take his widely used nomenclature into account, we named this tumor SMtTW: spontaneous (S) mammatropic or prolactin (Mt) tumor (T) in Wistar (W).

MATERIALS AND METHODS

Animals. The donors and host animals were rats of the WF/Ico strain. Since 1979, this strain has been inbred by more than 42 consecutive brother-sister matings at Ifsa Credo (69210, Saint Germain-sur-l’Arbresle, France) and then been perpetuated by full sibling matings. The animals were delivered to our laboratory at 1 mo of age. They were routinely housed 4 to 5 to a cage at 22–23°C. They were fed a standard rat diet (Sourirrat; Ifsa Credo) and had tap water ad libitum. The donor animals and hosts were females (n = 119). All the animals were free of hormonal treatment, namely, estrogen treatment.

Transplantations. The two primary tumors were removed from two 28-mo-old female WF/Ico rats (RF244 and RF330). These females were taken at random from 17- to 30-mo-old females bearing a spontaneous prolactinoma with high plasma prolactin levels. The primary and grafted tumors were removed by sterile technique, cut into fine pieces (size, 2 × 2 mm; weight, 5 mg), and slipped under the skin of the left leg and/or the kidney capsule of female host rats. The number of animals, the age of the hosts at the time of transplantation, as well as the duration of the graft at each passage are given in Table 1. All the tumors transplanted from the first in situ spontaneous prolactinoma (RF244) were called SMtTW₁. Similarly SMtTW₂ designated all the tumors transplanted from the second in situ prolactinoma (RF330), and so on. At the end of passage 4, 117 females were grafted with SMtTW₁ and SMtTW₂.

Bromocriptine Experiment. On passage 4 of SMtTW₂, at 4 mo of graft, 16 females were given injections daily, s.c., with 10 mg/kg of 2-bromoergocriptine, during 2 mo. Sixteen females without treatment served as control. PRL* measurements were performed before treatment (30 days, 3 and 4 mo of graft) and under bromocriptine (5 and 6 mo of graft). The animals were killed at 6 mo of graft.

Immunostaining for Hormones. Blood samples were taken under ketamine anesthesia in a carefully standardized manner before the graft, at

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2 To whom requests for reprints should be addressed, at Laboratoire d’Histo logie, Faculté de Médecine Alexis Carrel, Rue Guillaume Paradin, F-69372 Lyon, Cedex 8, France.

3 Unpublished observations.

4 The abbreviations used are: PRL, prolactin; GH, growth hormone; ACTH, adrenocorticotropic hormone; RIA, radioimmunoassay; PAS, periodic acid Schiff; hGH, human growth hormone; rGH, rat growth hormone; rPRL, rat prolactin; bLH, human luteinizing hormone; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases.
1- or 2-mo intervals after the graft and on the day of killing. Plasma PRL and GH were measured by double antibody RIA with the reagents kindly donated by the NIDDK. For the prolactin assay, the samples with high plasma levels were checked twice. The intraassay variability was 12.6%. The interassay variability was 11.1%. Immunoreactive ACTH was determined by RIA using a commercial kit (International C.I.S., Gif-sur-Yvette, France); the antibodies were directed against the 1-24 fragment.

Processing of Tumors for Morphological Studies. After decapitation, tumors were removed immediately, separated from renal tissue, measured, and weighed without blood. Few were photographed. For light microscopy, tumor fragments were fixed in Bouin-Holland HgCl₂ fluid. Histological observations were performed on slides stained with Masson’s trichrome, Herlant’s tetrachrome, and PAS-orange-G. Immunocytochemical studies were done by indirect immunoperoxidase, with or without streptavidin-biotin complex, and immunofluorescence. The duration of exposure to the primary antisera was 12 h, and the dilutions varied from 1/500 to 1/5000. The following rabbit antisera were used: anti-hGH and anti-hLH (prepared by B. Claustrat); anti-rPRL and anti-rβ,αACTH (prepared by M. P. Dubois); and anti-rGH and anti-rGH (prepared by Dr. Raiti, NIDDK). The specificity of the antisera and of the reaction was assessed by extinction of the reaction after (a) preabsorption of the antisera by excess homologous antigens, (b) unchanged reaction after preabsorption of anti-rGH and anti-rPRL antisera by heterologous antigens (rPRL and rGH, respectively), (c) absence of reaction when normal rabbit serum was substituted for the specific antibodies. For electron microscopy, tumor fragments were fixed in 2% glutaraldehyde in cacodylate buffer (pH 7.4, 100 mmol) and postfixed in 2% osmium tetroxide. They were embedded in Araldite. The ultrathin sections were examined with a JEOL-type 1200 EX electron microscope.

Morphological and Immunocytochemical Characteristics of SMtTW. Since the former were less hemorrhagic than were the SMtTW₁, thus, we certainly grafted a greater number of viable cells per fragment. Tumors were first palpable 6 mo after the graft under the kidney capsule. The curve of tumor growth was established for the SMtTW₁ (Fig. 1A). The tumor weights correspond to the weight of tumoral cells after removal of the kidney and without blood. The mean values of the tumor weight were significantly different between 6 and 12 mo (2.1 ± 0.42 g and 9.3 ± 3.29 g, respectively; P < 0.01) and between 8 and 12 mo (3.9 ± 0.75 g and 9.3 ± 3.29 g, respectively; P < 0.05). There was, however, no significant difference between 6 and 8 mo, and between 8 and 10 mo. At 8, 10, and 12 mo of graft, the tumor weight varied in a large range from animal to animal but not from passage to passage. At 6 mo of graft, the range was smaller (Fig. 1B). The tumor size was also variable. At 8 mo of graft, the smallest measured 1.2/1/0.4 cm, and the largest, 3/2.8/3 cm. The tumor growth of the SMtTW₂ was similar (results not shown).

Tumor Secretion

According to plasma RIA measurements, the tumors secrete prolactin only (Table 2). ACTH plasma levels were slightly elevated in 4 SMtTW₁-bearing rats after 8 mo of graft. Study of ACTH secretion at 1, 3, and 8 mo showed no increase with tumor growth. The curve of PRL secretion was studied at all the passages for SMtTW₁ (Fig. 2) and at passage 4 only for SMtTW₂ (Fig. 3). The plasma prolactin increased after 3 or 5 mo of graft.

Effects of Bromocriptine on Prolactin Secretion and Tumor Growth

At 4 mo of graft, just before treatment, the mean plasma PRL value was 537.9 ± 146.8 ng/ml. At 5 and 6 mo of graft, the plasma PRL concentration was <15 ng/ml in all treated rats versus 2783.2 ± 1247.6 ng/ml and 6656.3 ± 3358 ng/ml in the control group (Fig. 3). The antitumoral effect is evident in Fig. 4. The mean tumor weight of treated and control rats was 0.19 ± 0.09 g and 2.9 ± 1 g, respectively (Fig. 5).

Table 1 Transplantability of spontaneous prolactinomas in the female WF/Ico rat

<table>
<thead>
<tr>
<th>Passages</th>
<th>Grafted rats</th>
<th>Duration of graft (mo)</th>
<th>Success of graft (no. of takes/ no. of rats grafted)</th>
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<tr>
<td></td>
<td>n</td>
<td>Age (mo)</td>
<td>Kidney</td>
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<td>Passage 1</td>
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<td>SMtTW₁</td>
<td>1</td>
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<tr>
<td>SMtTW₂</td>
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<td>15</td>
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<td>Passage 2</td>
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<td>SMtTW₁</td>
<td>4</td>
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<td>12</td>
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<tr>
<td>SMtTW₂</td>
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<td>Passage 3</td>
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<tr>
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<td>6</td>
<td>6-8-10-12</td>
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<tr>
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<td>Passage 4</td>
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<td>2</td>
<td>6-8</td>
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<tr>
<td>SMtTW₂</td>
<td>58</td>
<td>2</td>
<td>4-6-8</td>
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SMtTW₁ and SMtTW₂ designate all the tumors transplanted from the first and the second in situ prolactinoma grafted.
Gross Inspection. When the tumors were grafted under the kidney capsule, they appeared ovoid and reddish. They were soft, friable, and often hemorrhagic. They were well circumscribed, easily distinguished, and removed from the kidney (Fig. 4). No metastases were observed. These tumors were considered as benign, except in one animal. This unique malignant tumor was very large, weighed 24 g, and invaded the kidney with a lymph node metastasis. The tumors grafted under the skin were pinkish and soft but never hemorrhagic.

Morphological Characteristics. By light microscopy, the cells were in diffuse arrangement with small capillaries and blood-filled spaces. They were agranular with Herlant's tetrachrome.
and PAS negative. Signs of secretory activity were conspicuous: large nucleoli and clear juxtanuclear Golgi areas. Mitoses were often observed. Except in one animal, the limit between the surrounding tissue (kidney or adipose tissue) was clear without invasion. Some areas of necrosis and fibrosis were observed in this tumor and in a few other tumors.

By electron microscopy, the cells were well differentiated (Fig. 6). The polymorphous secretory granules were numerous and located in the Golgi area. They measured 70 to 130 nm (mean diameter, 100 nm). Misplaced exocytosis was often observed. The Golgi complexes and the rough endoplasmic reticulum were well developed. Some mitochondria and liposomes were also noted.

**Immunocytochemical Characteristics.** By immunocytochemistry, the reaction was strongly positive in all the cells with the two anti-rPRL antisera (Fig. 7). The reaction was often located in the Golgi area. It was abolished after preabsorption of the antisera with rPRL antigens and remained unchanged after preabsorption with rGH. In all tumors except one, no reaction was observed with anti-rGH antiserum. In the somatoprolactin tumor, the reaction with anti-rGH antiserum was abolished after preabsorption of this antiserum with rGH. The percentage of PRL- and GH-immunoreactive cells was, respectively, 80% and 20%. In all tumors, no reaction was observed with the other antisera tested (anti-\(\beta\)ACTH and anti-hLH antisera).

**Miscellaneous**

Except in the unique rat bearing a somatoprolactin tumor of which the body weight was 348 g (mean body weight in adult female WF/Ico rat, 250 g), no variation in body weight was observed in the SMtTW-bearing rats. A hyperplasia of the mammary gland was noted in all female rats. Adrenal glands were normal. In SMtTW-bearing rats, the adrenal weights were normal even in the 4 animals with slightly increased adrenal weights (results not shown). In SMtTW tumors, at passage 4, the mean adrenal weights were 28.5 ± 2 mg in the control group and 31.8 ± 2.2 mg in the bromocriptine-treated group (mean adrenal weight in the strain, 30.1 ± 2 mg). The mean tumor weights were 2.9 ± 1 g and 0.19 ± 0.09 g, respectively (Fig. 5). In the control group, an analysis of the adrenal weight normalized per tumor size indicated that there is no correlation between the adrenal and the tumor weights (results not shown).

**DISCUSSION**

We present here the first description of a spontaneous transplantable tumor in the rat. In 1960 and 1973, Furrth and coworkers (1, 2) noticed that pituitary tumors which occur spontaneously can be transplanted to untreated histocompatible hosts without endocrine or other manipulation. To our knowledge this suggestion has not been followed up. Currently, in our laboratory, six such tumors are at different serial passages (passages 2 to 9), and the percentage of graft success is 100% under the kidney capsule and 20% under the skin of the leg. Thus, it can be said that, if the spontaneous prolactinomas of the WF/Ico rats are grafted under the kidney capsule of consanguineous rats, all such tumors are found to be transplantable. No immunological reactions were observed. These pituitary tumors are not immunogenic. Some SMtTW subcutaneously grafted into nude female rats. They did not grow more rapidly than in the WF/Ico consanguineous rat strain (results not shown).

Since it is known that spontaneous prolactinoma is more frequent in female rats than in males, we first transplanted the SMtTW in females. Recently we found that it also grows in the male, but more slowly (results not shown). This fact indicates that the SMtTW may be considered to be estrogen sensitive. But the transplantation of the spontaneous tumor does not require estrogen treatment of the host. The age of the host does not play a major role. If the tumor is grafted to a host of 10 mo as we did in passage 1 of SMtTW, an in situ prolactinoma can grow simultaneously with the grafted tumor. Thus, the prolactin plasma values are not the marker of the grafted tumor only. Therefore, all the host animals were 2 mo old, and they were killed before 12 mo. At this age, spontaneous prolactinomas are exceptional (17).

Throughout the first four passages studied, the SMtTW tumors remained identical. No variations of tumor growth rate and no histological modifications were noted. During the period studied (6 to 12 mo), the growth rate of the SMtTW tumor was slow. Although the tumor growth over the first 6 mo of graft was not widely studied, it may be thought that it was also slow because the tumors grafted under the kidney capsule were first palpable at 6 mo. Six SMtTW tumors removed after 2 and 4 mo of graft weighed a mean of 7 mg and 370 mg (results not shown). The small size of the tumors may also be deduced from the low values of plasma prolactin concentration during the first 4 mo. Between 4 and 6 mo of graft the plasma prolactin concentration increased rapidly, the weights of the tumors were significantly different, and the dispersion of the values at 6 mo of graft was moderate. Thus, this period must be chosen to test the antitumoral effect of a drug, as done in the bromocriptine experiment.

Since the tumors grew slowly and no invasion of surrounding tissues nor metastases were observed, the SMtTW tumor may be considered to be benign. This differentiates it from most other transplantable tumors which grow rapidly (11). However, one tumor was considered as malignant. Its PRL secretion seemed low with respect to tumor size (4400 ng/ml for 24 g). The plasma levels of GH and ACTH were normal.

According to the morphological characteristics of the cells which look like prolactin cells, the SMtTW tumor may be considered as a well-differentiated tumor with features of exocytosis. These ultrastructural characteristics differ from the
MtTF₄ and MtTW₁₀ tumors in which most cells were without granulations (10, 11). According to the hormonal plasma values, the immunocytochemical results, the absence of variation in the body weight, and the normal surrenal weight of the hosts, all the SMtTW tumors, with one exception, secrete prolactin only. The prolactin secretion increased with the duration of graft and with the tumor size and reached very high levels (7000 ng/ml). The prolactin may be considered as a marker of tumor growth. It is biologically active because it induces mammary gland hyperplasia.

The SMtTW tumor differs from the induced transplantable tumors which secrete GH and PRL (MtTW₅, MtTW₁₅) or GH, PRL, and ACTH (MtTF₄). The multisecretion became apparent during serial transplantations (6). In the SMtTW tumor, at passage 3 one animal exhibited an excess of weight, and the grafted tumor was somatoprolactinic. Unfortunately the tumor was not grafted. This problem concerning the variability of secretion requires further investigation. Contrary to other transplantable tumors (8), our experiment showed that bromocriptine, a dopamine agonist, is effective in reducing prolactin.

Fig. 6. The SMtTW tumor is a sparsely granulated prolactinoma (A, × 7,500) with well-developed Golgi area and rough endoplasmic reticulum (B, × 11,000) and many granule extrusions (C, × 30,000).
secretion and inhibiting tumor growth. Recently, Judd et al. (19) isolated a cell line which secretes only PRL from the 731s5a tumor, a carcinogen-induced PRL-, GH-, and ACTH-transplantable rat pituitary tumor. This model is interesting but differs from the SMtTW model. In the MMQ cell line, the grafted tumors grow rapidly; the cells are undifferentiated. In vivo and in vitro, the secretion of PRL is low. In culture, the dopamine only attenuates the secretagogue-induced PRL release and does not decrease the basal secretion.

As recent studies suggested (Refs. 4 and 5; Footnote 3), the pituitaries of estrogen-treated rats are not tumoral but only hyperplastic in a great majority of animals. This debatable point must be further discussed and developed. Two pieces of evidence can be put forward: (a) the “tumors” of estrogen-treated rats are not transplantable without estrogen treatment of the hosts and (b) the weight of the pituitary falls abruptly and returns to normal after the removal of estrogen pellets. A spontaneous regression of a pituitary tumor has never been shown in either humans or animals. We showed in this work that spontaneous prolactinomas of the rat are transplantable without treatment of consanguineous hosts.

Contrary to the in situ spontaneous prolactinoma of the rat, the SMtTW tumor is a very easy model to use; the graft is easy, rapid, and successful. It is possible to have a large number of SMtTW-bearing animals together. The tumors are accessible. Given the tumor sizes, many studies may be performed. The tumor growth is such that the antitumor effects of new drugs may be tested. This model may be used by researchers interested in the subcellular mechanisms of prolactin secretion and by endocrinologists working on the effects of prolactin in the reproductive system and the lactation.

In light of our knowledge of human prolactinoma (20, 21), we consider the SMtTW tumor to be very close to the human pathology because: (a) the primary tumor was spontaneous; (b) at the first passages the tumors are benign and grow slowly; (c) they secrete prolactin only; (d) they have the same morphological characteristics as the human prolactinoma; (e) they are estrogen sensitive; and (f) their secretion and their growth are inhibited by a dopamine agonist. For studying the factors of promotion and progression of pituitary tumors, this model seems more adequate than the other transplantable tumors which are induced and malignant.

We hope that the SMtTW tumor will be considered as a valuable model for research on pituitary tumors, but no single tumor system is a perfect model. Researchers have therefore to work on different models each of which is appropriate for investigating specific problems.

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