

# Use of micro-positron emission tomography with $^{18}\text{F}$ -fallypride to measure the levels of dopamine receptor-D2 and $^{18}\text{F}$ -FDG as molecular imaging tracer in the pituitary glands and prolactinomas of Fischer-344 rats

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**Abstract:** Dopamine receptor-D2 (DRD2) is the most important drug target in prolactinoma. The aim of this current study was to investigate the role of using micro-positron emission tomography (micro-PET) with  $^{18}\text{F}$ -fallypride and  $^{18}\text{F}$ -fluorodeoxyglucose ( $^{18}\text{F}$ -FDG) as molecular imaging tracer in the pituitary glands and prolactinomas of Fischer-344 (F344) rats and detect the difference of the levels of DRD2 in the pituitary glands and prolactinomas of F344 rat prolactinoma models. Female F344 rat prolactinoma models were established by subcutaneous administration of 15 mg  $17\beta$ -estradiol for 8 weeks. The growth of tumors was monitored by the small-animal magnetic resonance imaging and micro-PET. A series of molecular biological experiments were also performed 4 and 6 weeks after pump implantation. The micro-PET molecular imaging with  $^{18}\text{F}$ -fallypride revealed a decreased expression of DRD2 in F344 rat prolactinoma models, but the micro-PET molecular imaging with  $^{18}\text{F}$ -FDG presented an increased uptake in the prolactinoma compared with the pituitary gland. A decreasing trend of levels of DRD2 in F344 rat prolactinoma models was also detected by molecular biological experiments. From this, we can conclude that micro-PET with  $^{18}\text{F}$ -fallypride and  $^{18}\text{F}$ -FDG can be used to assess tumorigenesis of the prolactinomas in vivo and molecular imaging detection of DRD2 level in prolactinoma may be an indication of treatment effect in the animal experiment.

**Keywords:** pituitary prolactin, dopamine agonists, prolactinoma

## Introduction

Prolactinomas are the most common hormone-secreting pituitary tumors.<sup>1</sup> Dopamine agonists (DAs) are the first-line treatment for prolactinomas, but treatment responses are often variable. The majority of prolactinoma patients treated with DAs respond with a normalization of the pituitary prolactin (PRL) levels and a reduction in tumor volume. However, more than 10% of prolactinoma are resistant to DAs.<sup>2,3</sup> Studies of in vitro cell preparations show that the dopamine receptor-D2 (DRD2) of resistant tumors are decreased in number but have normal affinity.<sup>4</sup> The PRL secretory cells predominantly express the DRD2.<sup>5,6</sup> Evaluation of the DRD2 level in prolactinoma based on molecular imaging could help determine whether drug treatment with DAs is effective or not. But little research has been done to detect the DRD2 level in pituitary tumors by using noninvasive molecular imaging methods.

Positron emission tomography (PET) is a valuable method for the diagnosis and staging of tumorigenesis as well as for monitoring the therapeutic effectiveness of various treatment modalities. One of direct binding probes targeting DRD2/dopamine receptor-D3 imaging agent is  $^{18}\text{F}$ -fallypride and one of indirect imaging probes is  $^{18}\text{F}$ -fluorodeoxyglucose ( $^{18}\text{F}$ -FDG). Using  $^{18}\text{F}$ -FDG in PET imaging over the last 2 decades increased the detection of incident of the pituitary tumors.<sup>7</sup> Among the PET D2/D3 antagonist radioligands, three have been utilized in studies with human subjects and have continued to appear in recent literature. These are  $^{11}\text{C}$ -raclopride,  $^{11}\text{C}$ -FLB457, and  $^{18}\text{F}$ -fallypride;<sup>8</sup>  $^{18}\text{F}$ -fallypride is suitable for studying DRD2/dopamine receptor-D3 in both the human and the nonhuman brain. During the last decade,  $^{18}\text{F}$ -fallypride has led to considerable interest from clinical and pharmacological research perspectives.<sup>9</sup>

The chronic diethylstilbestrol or estrogen treatment induces prolactinoma in rat models. Development of prolactinoma is highly dependent on the rodent strain, with the Fischer-344 (F344) rats being the most susceptible.<sup>10</sup> To further elucidate the prolactinoma tumorigenesis, the estrogen-induced F344 rat prolactinoma models were successfully established. Piroli et al<sup>11</sup> described that the female F344 rats more easily induced prolactinoma than male F344 rats and female F344 rats implanted with a single 20 mg diethylstilbestrol pellet usually developed pituitary tumors within 30–45 days. Heaney et al<sup>12</sup> reported that induced by  $17\beta$ -estradiol for 4 weeks, all ovariectomized female F344 rats developed prolactinoma, associated with increased PRL messenger RNA (mRNA) levels, pituitary weight, and serum PRL levels.

Our goal in this study was to evaluate the feasibility of  $^{18}\text{F}$ -fallypride and  $^{18}\text{F}$ -FDG to visualize the distribution and the changed level of DRD2 in F344 rat prolactinoma models compared with the pituitary glands. Small-animal magnetic resonance imaging (MRI) was also used for determining the volume of the prolactinomas in rat models. Polymerase chain reaction (PCR) and Western blot were used to evaluate the mRNA and protein expression of DRD2. Our research will provide basic evidence to the in-depth research in rat prolactinoma models.

## Materials and methods

### Ethics statement

All experimental protocols followed the National Institutes of Health guidelines for the use and care of animals and were approved by the Institutional Review Board and the Animal Care and Use Committee of Capital Medical University. Animal execution was performed according to Capital Medical University guidelines.

## Animals

One hundred 4-week-old female F344 rats (60–70 g of bodyweight) were obtained from Vital River Laboratories (Beijing, People's Republic of China). The animals were housed under controlled conditions of light (lights on from 7 am to 7 pm) and temperature (20°C–25°C), with water and food available ad libitum. One week later, after all of the rats adapted to the environment, the ovariectomy was performed. Penicillin was administered intramuscularly for 3 days. One week after the surgery and wound healing, the animals underwent chronic estrogen treatment using the following regimen: ALZET osmotic pumps (model 2006, DURECT Corporation, Cupertino, CA, USA) containing 15 mg of  $17\beta$ -estradiol (Sigma-Aldrich Co., St Louis, MO, USA) in 50% (v/v) glycerol/50% (v/v) dimethylsulfoxide solution were implanted subcutaneously to induce prolactinoma. Control animals were implanted with pumps containing only vehicle. The animals were divided into two groups: 50 rats as control group and 50 rats as tumor group.

## MRI scanning

MRI was performed using a 7.0T (Bruker Optik GmbH, Ettlingen, Germany) small-animal MRI machine. On follow-up 4 and 6 weeks after the pump implantations, small-animal MRI was used to detect the growth of the prolactinomas in all rats of tumor and control groups. T1-weighted spin-echo images were obtained in the coronal and sagittal planes at 3 mm sections. T1-weighted-enhanced images were then obtained after tail vein intravenous administration of 100  $\mu\text{L}$ /25 g (rat weight) of Gadospin<sup>TM</sup> M Gd-DTPA (Miltenyi Biotec, Bergisch Gladbach, Germany) as a contrast-enhanced agent.

## $^{18}\text{F}$ -fallypride and $^{18}\text{F}$ -FDG synthesis

The PET tracers,  $^{18}\text{F}$ -fallypride and  $^{18}\text{F}$ -FDG, were prepared by the reaction of  $^{18}\text{F}$  with precursors, according to published methods.<sup>13,14</sup> The decay corrected radiochemical yield was ~24%; the specific radioactivity was greater than 100 TBq/mmol at the moment of injection, and the radiochemical purity was greater than 98%. The  $^{18}\text{F}$ -fallypride and  $^{18}\text{F}$ -FDG solutions had a pH of 6.0–7.0.

## PET scanning

On follow-up 4 and 6 weeks after the pump implantations, the pituitary masses had substantially increased in size. PET was performed at the same time intervals using  $^{18}\text{F}$ -fallypride, which targets to DRD2 mainly expressed in pituitary and prolactinoma. In addition,  $^{18}\text{F}$ -FDG PET imaging monitoring in the two groups was performed next day.

According to the groups, all rats were fasted for more than 2 hours prior to the test. PET was performed using a micro-PET scanner (Inveon; Siemens, Munich, Germany) that was equipped with a micro-PET Manager for data acquisition in a list mode and ASI Pro VM™ software for preparing sinograms and image construction. Thirty minutes after tail vein injections of 0.5 to 0.6 mCi of <sup>18</sup>F-fallypride or <sup>18</sup>F-FDG, images were obtained using the small-animal PET scanner with a 10-minute emission scan. Two experienced nuclear medicine doctors evaluated the <sup>18</sup>F-fallypride and <sup>18</sup>F-FDG PET images on a high-resolution computer screen. Regions of interest were also drawn over the pituitary in the control group and the prolactinoma in the tumor group. For quantitative analysis, regions of interests on the micro-PET images were drawn by a single individual to minimize variation in region selection. Tracer uptake was expressed as percentage of injected radioactivity dose per gram of tissue (%ID/g).

## Experimental protocol and preparation of tissues

At the interval of 4 or 6 weeks, female F344 rats were anesthetized by intraperitoneal injection using 10% chloral hydrate 400 mg/kg in each group. Then, the tail vein blood was collected, and the serum was separated by centrifugation and stored at -80°C until the measurement of serum PRL and the pituitary tissue was removed, weighed, measured, and photographed. Each group had ten rats' fresh pituitary glands that were carefully isolated and kept in liquid nitrogen until the rest of the PCR (n=10 in each group at one time interval of 4 weeks (Ws)/6Ws) and Western blot procedures (n=10 in each group at one time interval of 4Ws/6Ws). Another five rats in each group were executed, and the specimen of pituitary or tumor was collected for paraffin sections and frozen sections (n=5 in each group at one time interval of 4Ws/6Ws).

## Measurement of PRL in rat serum

Rat serum PRL concentrations were determined using an ELISA kit (Pulilai, Beijing, People's Republic of China) based on the double antibody method. The lower limit of the assay sensitivity was 1.0 ng/mL. Inter- and intra-assay coefficients of variations were both less than 10%.

## Reticular fiber staining, hematoxylin and eosin staining, and VEGF immunohistochemistry staining

Paraffin-embedded F344 rat pituitary tissues were cut into sections that were routinely stained with hematoxylin and eosin staining by using the hematoxylin and eosin staining kit (Beijing Yili Fine Chemicals Co. Ltd., Beijing, People's

Republic of China), reticular fiber staining (Beijing Yili Fine Chemicals Co. Ltd.), and vascular endothelial growth factor (VEGF) immunohistochemistry staining (VEGF antibody, ab46154; Abcam, Cambridge, UK). Then, the sections were observed under a Nikon inverted microscope. Then, magnification 400× fields were used to compare the differences between the control and tumor groups.

## Immunofluorescence

The DRD2 and PRL expression of the pituitary glands and the prolactinomas was analyzed using immunofluorescence (PRL antibody, 1:400, Santa Cruz Biotechnology, Inc., Dallas, TX, USA; DRD2 antibody, 1:100, ab32349, Abcam). Sections were examined on a fluorescence microscopy (Leica, Heidelberg, Germany) and a C1 Plan Apo ×63/5 oil confocal laser scanning system, LSM 510 META (Leica).

## Quantitative real-time PCR

Total RNA was extracted from pituitary tissue using Trizol (Thermo Fisher Scientific, Waltham, MA, USA) according to the manufacturer's recommendations. For this experiment, 4 μg of total RNA was treated with an M-MLV reverse transcription kit (Thermo Fisher Scientific) according to the manufacturer's recommendations. cDNA was diluted with DRD2 Taqman® gene expression assay (ID: Rn00561126\_m1, DRD2, Thermo Fisher Scientific), RNase-free water, and Taqman® gene expression Master Mix (P/N 4369016, Thermo Fisher Scientific) using an ABI 7500 HT Real-Time PCR System (Thermo Fisher Scientific) according to the manufacturer's recommendations. PCR was amplified in duplicates and three independent experiments using a Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH) Taqman® gene expression assay (ID: Rn01775763\_g1, GAPDH, Thermo Fisher Scientific) as endogenous controls. PCR amplification was performed in 96-well optical reaction plates for 40 cycles, with each cycle at 95°C for 15 seconds and 60°C for 1 minute. Fold changes were calculated using the 2<sup>-ΔΔCt</sup> method.

## Western blot

The pituitary tissue was washed once in phosphate-buffered saline and lysed in RIPA lysis buffer. The pituitary tissue samples from the rat tumor and the control groups were cut into small pieces and homogenized in 100 μL lysis buffer containing protease inhibitors (Pulilai). The concentration of protein was determined by BCA (Pierce, Rockford, IL, USA). Aliquots (40 μg) were separated on a 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis and transferred to polyvinylidene fluoride membranes. The nonspecific binding of immunoproteins was blocked with 5% nonfat

dry milk for 1 hour at room temperature. After blocking, the membranes were incubated with primary antibodies overnight at 4°C. The primary antibody that recognizes DRD2 was ab32349 (1:1,000), an anti-DRD2 antibody purchased from Abcam. The membrane was rinsed in Tris-buffered saline containing 0.1% polysorbate 20 minutes followed by 1 hour of incubation with horseradish peroxidase-conjugated secondary antibody (rabbit anti-goat IgG) at room temperature. An enhanced chemiluminescence detection solution was applied (Santa Cruz Biotechnology, Inc.). The protein bands were visualized on X-ray films. The optical density of the protein bands was quantified using ImageJ (National Institutes of Health, Bethesda, MD, USA) and standardized to GAPDH (1:1,000, Sigma-Aldrich Co.).

### Statistical analysis

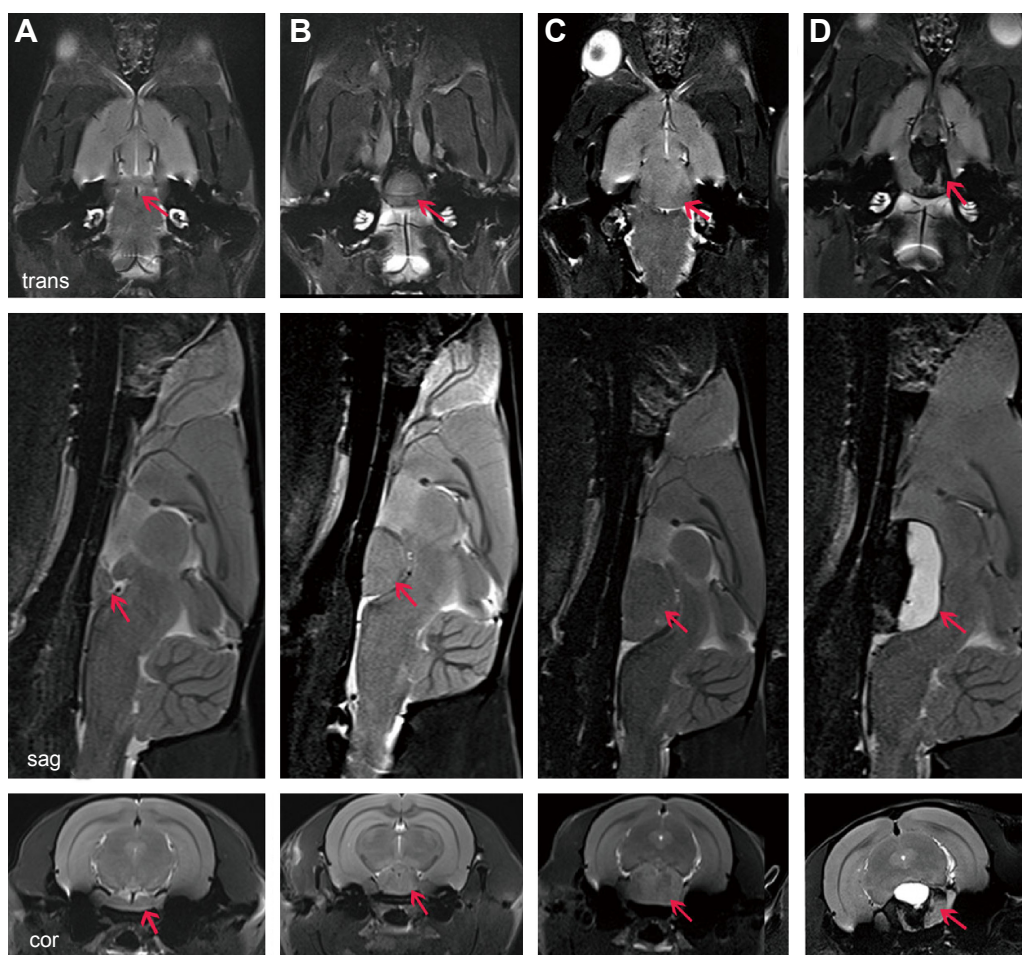
The results are expressed as the mean  $\pm$  standard deviations. All the tests were performed in triplicate and repeated three times. Statistical analyses were performed using one-way

analysis of variance or the Student's *t*-test. A level of  $P < 0.05$  was considered to be statistically significant (SPSS Inc., 17.0, Chicago, IL, USA).

## Results

### Tumor visualization of MR imaging

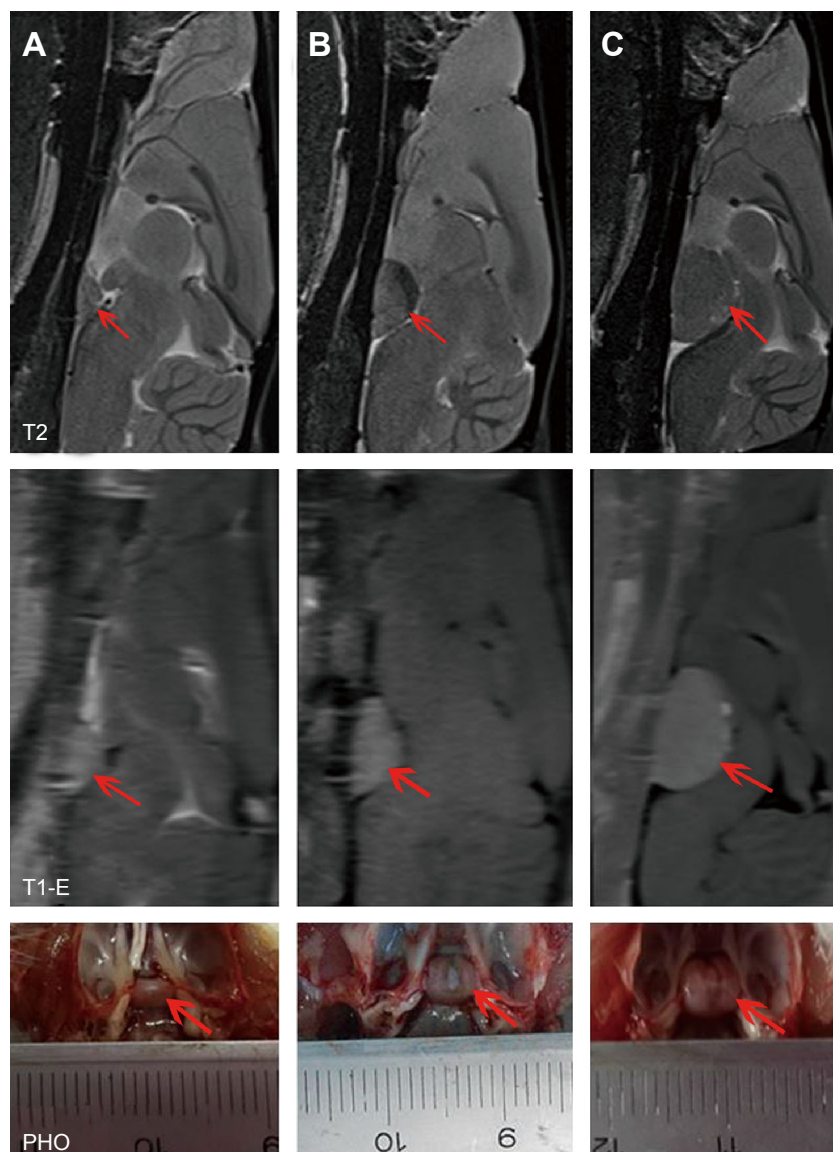
In our study, 17 $\beta$ -estradiol-induced prolactinoma developed in 100% of the female F344 rats. Tumors were clearly visible on the MRI scans (Figure 1). Four weeks after subcutaneously implanted the capsule, it can be seen that the prolactinomas were obviously larger than the normal pituitary, and the surrounding brain tissues were oppressed by the tumor (Figure 1A and B). Six weeks later, the prolactinomas grew rapidly, and the MR imaging revealed that the prolactinoma were much bigger (Figure 1C). With stimulation for 8 weeks, pituitary apoplexy developed in some cases (Figure 1D), and the incidence of pituitary apoplexy was high (70%). We also performed imaging in the control and tumor groups by using Gadospin™ M Gd-DTPA contrast-enhanced agent. As shown



**Figure 1** MR images of control and tumor group rats.

**Notes:** Small-animal MR images of control group rats (A) and tumor group rats with induced pituitary tumor at 4 weeks (B) and tumor at 6 weeks (C) and tumor group rats with pituitary apoplexy at 8 weeks (D). Red solid arrows indicate normal pituitary or prolactinoma.

**Abbreviations:** trans, transverse view; sag, sagittal view; cor, coronal view; MR, magnetic resonance.



**Figure 2** Enhanced MR images and photos of control and tumor group rats.

**Notes:** Small-animal MR images of normal pituitary (A) and induced pituitary tumor at 4 weeks (B) and 6 weeks (C), and T2-weighted image (T2) and contrast-enhanced T1-weighted image (T1-E). Red solid arrows indicate normal pituitary or prolactinoma.

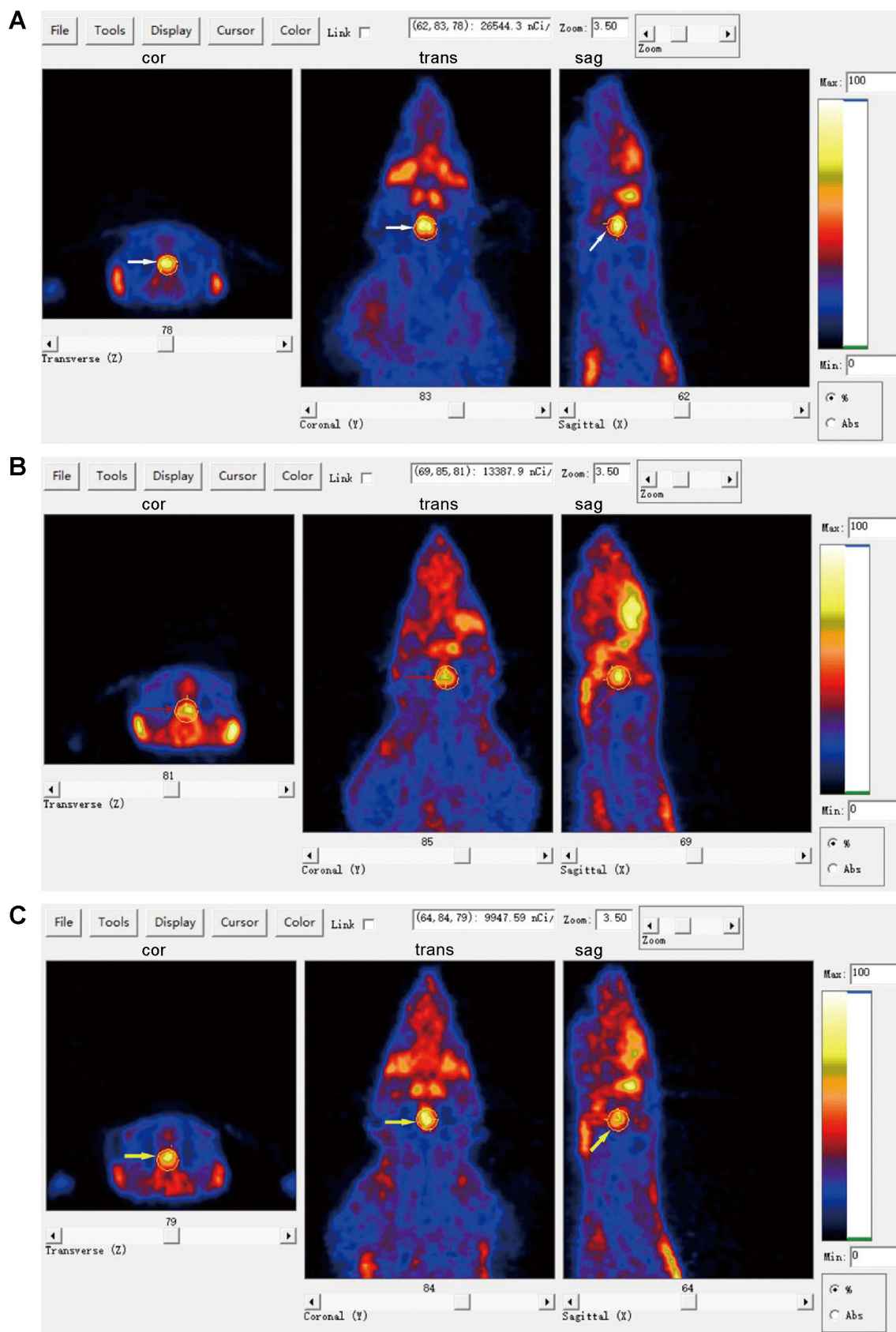
**Abbreviations:** MR, magnetic resonance; PHO, photo view.

in Figure 2, the larger pituitary tumors were clearly enhanced with the same condition that tumors at 6 weeks (Figure 2C) were much larger than the tumors at 4 weeks (Figure 2B) and normal pituitary (Figure 2A).

### Uptake of $^{18}\text{F}$ -fallypride and $^{18}\text{F}$ -FDG in female F344 rats between control and tumor groups

Based on the imaging data and statistical analysis, when the prolactinomas were successfully induced, the volume of the rats' prolactinomas in 4 and 6 weeks was larger than that of the controls. The  $^{18}\text{F}$ -fallypride is a molecular imaging tracing agent that targets to DRD2. Based on the  $^{18}\text{F}$ -fallypride PET

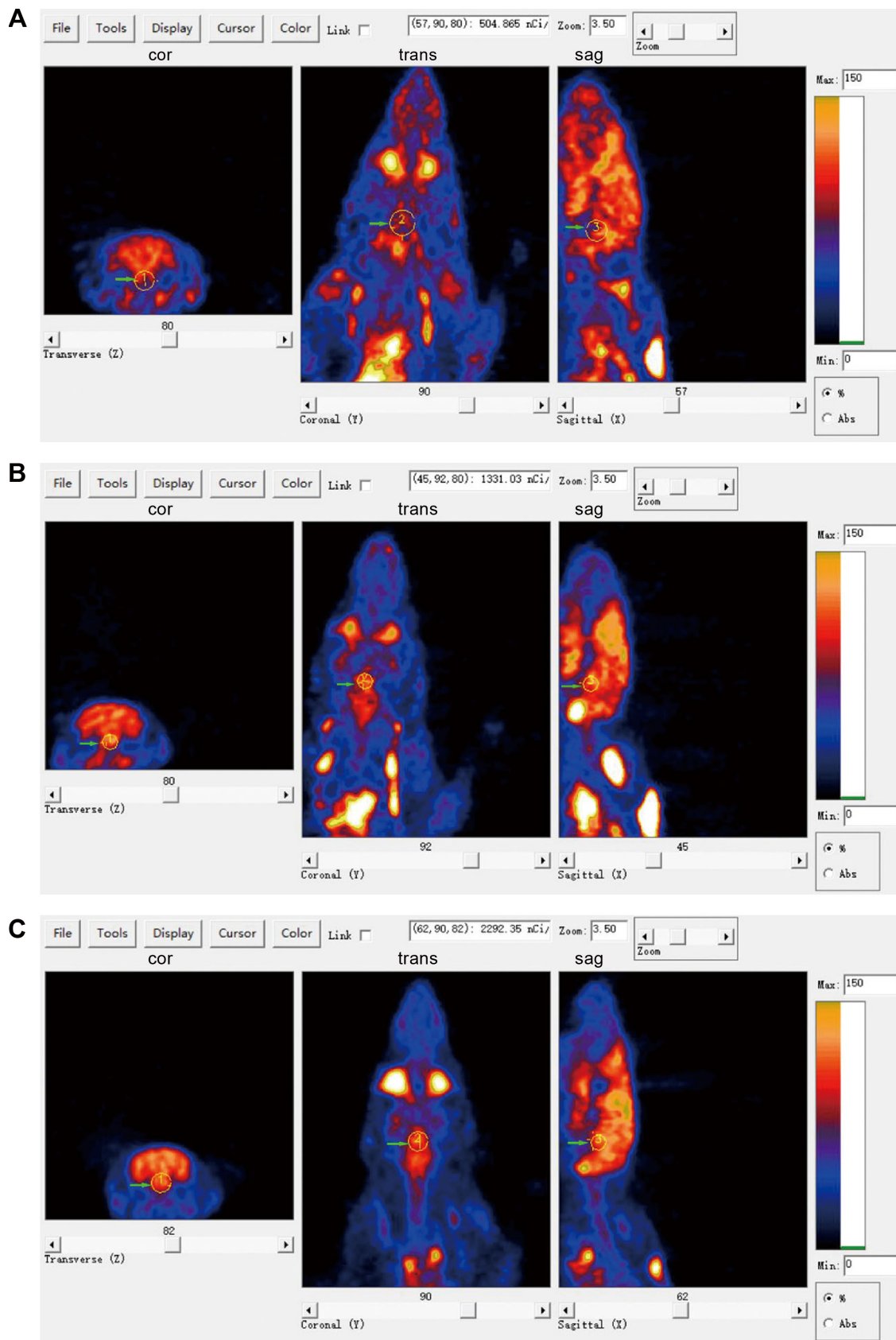
imaging, the SUVmax of the DRD2 in the pituitary regions of interest of the control group (Figure 3A) was higher than that of the tumor group at 4 and 6 weeks (Figure 3B and C). This indicates that the expression of DRD2 decreased with the prolactinomas induced by  $17\beta$ -estradiol. Based on the  $^{18}\text{F}$ -FDG imaging (Figure 4), the %ID/g in the tumor groups at 6 weeks was significantly higher than the control, which indicates that the metabolism and blood perfusion were high in the rat prolactinoma models, but it is not statistically significant between the control group and tumors at 4 weeks ( $P>0.05$ ). The statistical analysis of the %ID/g in different groups and with different tracer agents is summarized in Figure 5, and a value of  $P<0.05$  was considered to be statistically significant.



**Figure 3** PET images with  $^{18}\text{F}$ -fallypride of control and tumor group rats.

**Notes:**  $^{18}\text{F}$ -fallypride small-animal PET images of a control group rat (A) and tumor group rat with induced pituitary tumor at 4 (B) and 6 weeks (C). Solid arrows indicate normal pituitary or prolactinoma.

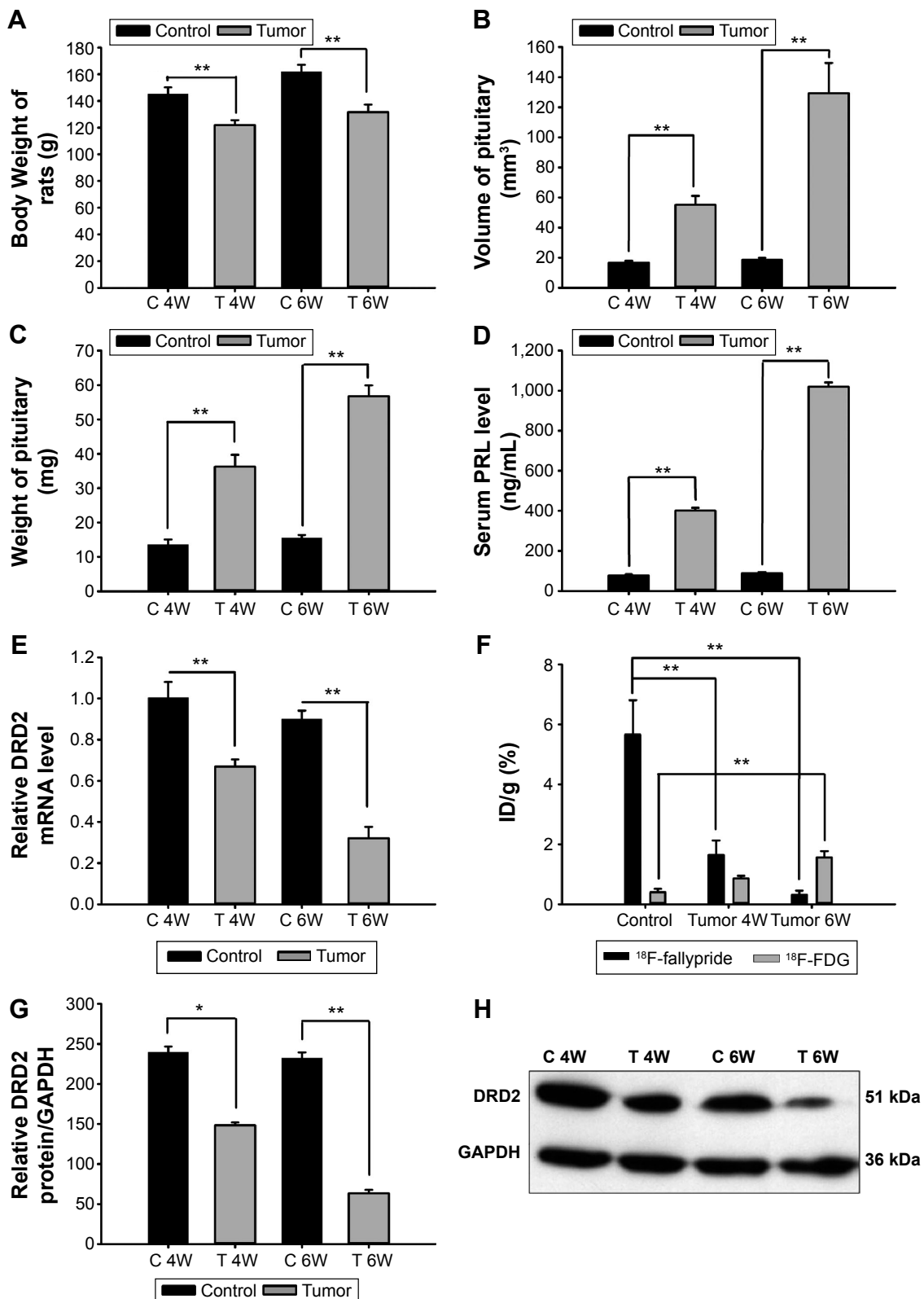
**Abbreviation:** PET, positron emission tomography.



**Figure 4** PET images with  $^{18}\text{F}$ -FDG of control and tumor group rats.

**Notes:**  $^{18}\text{F}$ -FDG small-animal PET images of control group rats (**A**) and tumor group rats with an induced pituitary tumor at 4 (**B**) and 6 weeks (**C**). Solid arrows indicate normal pituitary or prolactinoma.

**Abbreviations:** PET, positron emission tomography;  $^{18}\text{F}$ -FDG,  $^{18}\text{F}$ -fluorodeoxyglucose.



**Figure 5** The statistics of differences between the control and tumor groups.

**Notes:** (A) Weight of the rat in the control and tumor groups; (B) the volume of the rat pituitary in the control and tumor groups; (C) weight of the rat pituitary in the control and tumor groups; (D) the serum PRL levels in the control and tumor groups; (E) relative mRNA expression of DRD2 in the control group, 4-week prolactinomas, and 6-week prolactinomas; (F) the biodistribution of <sup>18</sup>F-fallypride and <sup>18</sup>F-FDG in the pituitary, and the %ID/g of the control and tumor groups. (G and H) Relative expression of DRD2 protein relative to GAPDH based on Western blotting in the control group, 4-week prolactinomas, and 6-week prolactinomas. \**P*<0.05 or \*\**P*<0.01 was statistically significant.

**Abbreviations:** mRNA, messenger RNA; DRD2, dopamine receptor-D2; GAPDH, Glyceraldehyde-3-Phosphate Dehydrogenase; PRL, prolactin; %ID/g, percentage of injected radioactivity dose per gram of tissue; W, week; <sup>18</sup>F-FDG, <sup>18</sup>F-fluorodeoxyglucose.



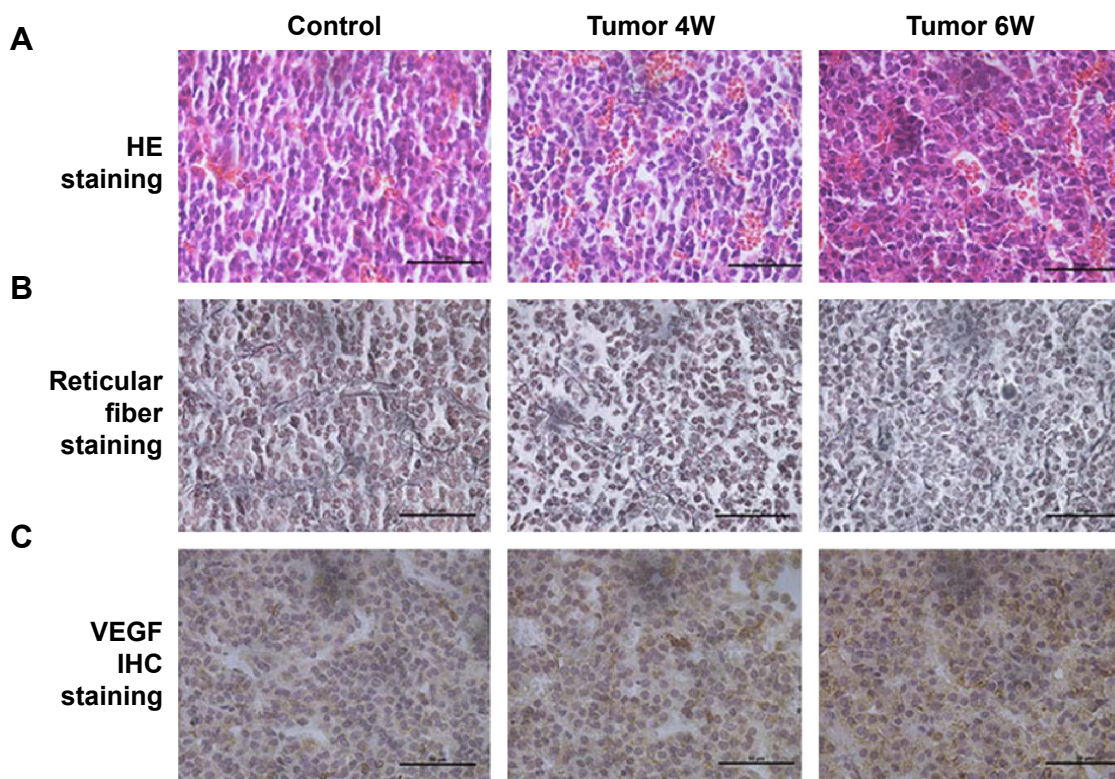
## The different histology, immunofluorescence, and serum PRL levels between the control and tumor groups

The hematoxylin and eosin staining results are shown in Figure 6A. The control group displayed cord-like arrangement of the groups of pituitary gland cells and sinus-like capillaries. Eosinophils are more numerous, larger, and had round nuclei and red-stained cytoplasm. In the experimental group, the pituitary tumor cells have abnormal morphology, disordered structure, differed in shape and size, and exhibited nuclear atypia, with unclear nuclear staining and structure, and violet-stained cytoplasm. The reticular fiber staining shows (Figure 6B): the control group display clear and complete reticular fibers, pituitary tumor cells of the tumor group have unclear and incomplete reticular fibers and the reticular fibers are fewer in number. Figure 6C shows the expression of VEGF is higher in the tumor groups than the control, and the VEGF expression in the 6-week tumor group is higher than that in the 4-week group.

The immunofluorescent staining confirmed the down-regulation of DRD2 and PRL in the rats' prolactinomas. As shown in Figure 7A and C, high DRD2 expression was

observed in the cells from the normal pituitary, whereas low DRD2 expression was observed in the rats' prolactinoma cells. Distinct differences of the DRD2 immunofluorescent staining were found between the control group and 6-week tumor group. Figure 7B and D shows that a high PRL expression was observed in the cells from the prolactinoma. The level of serum PRL was measured by using ELISA 4 and 6 weeks after  $17\beta$ -estradiol induction. Figure 5D shows the serum PRL level was significantly increased compared to the control group and obviously a difference was found between the 4- and 6-week tumor group. The serum PRL level results of the ELISA were consistent with the immunofluorescence analysis.

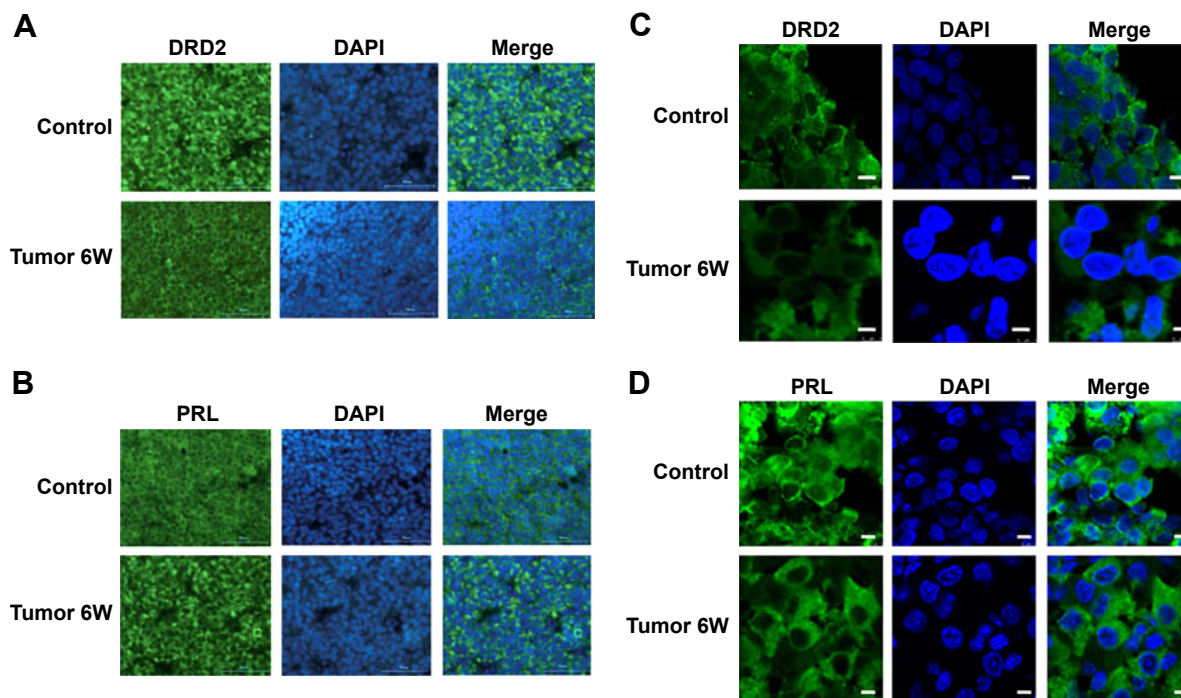
Control rats exhibited shiny fur, body hypertrophy, and good appetite. Experimental rats were small in size, slim, had dull fur, and displayed hair removal behavior and poor appetite. We can conclude that chronic  $17\beta$ -estradiol stimulation lowered the growth rate in rats. The average weights of the different groups were statistically analyzed as shown in Figure 5A. Pituitary volume and weight changed as shown in Figure 5B and C.



**Figure 6** The results of molecular biology experiments between control and tumor groups.

**Notes:** (A) HE immunohistochemical staining of the control group rats with normal pituitary and tumor group rats with an induced prolactinoma at 4 weeks and 6 weeks. (B) Reticular fiber staining of the control group rats with normal pituitary and tumor group rats with an induced prolactinoma at 4 and 6 weeks. (C) VEGF IHC staining of the control group rats with normal pituitary and tumor group rats with an induced prolactinoma at 4 and 6 weeks. Scale bar, 50  $\mu$ m.

**Abbreviations:** IHC, immunohistochemistry; HE, hematoxylin and eosin; VEGF, vascular endothelial growth factor; W, week.



**Figure 7** Expression of DRD2 (A, C) and PRL (B, D) in the control and 6 weeks tumor groups.

**Notes:** Immunofluorescence staining for DRD2 (green) and PRL (green) and the nucleus (DAPI, blue) were performed; the results show the significant downregulation of DRD2 and upregulation of PRL in the 6 weeks tumor group. (A, B): scale bar, 50  $\mu$ m; (C, D): scale bar, 5  $\mu$ m.

**Abbreviations:** DRD2, dopamine receptor-D2; PRL, prolactin; DAPI, 4',6-diamidino-2-phenylindole; W, week.

## The results of PCR and Western blot detection

We also detected the DRD2 mRNA levels by real-time PCR analysis of normal pituitary gland and prolactinoma; they were significantly lower in the prolactinoma (Figure 5E) whereas there is no significant difference between the tumor groups at 4 and 6 weeks. In addition, the DRD2 protein levels in the prolactinoma were significantly decreased compared to the control group. These findings showed that  $17\beta$ -estradiol-induced DRD2 downregulated in prolactinomas (Figure 5G and H). The PCR and Western blot results further confirmed that  $17\beta$ -estradiol induces prolactinoma in female F344 rats and suppresses DRD2 expression through specific molecular mechanisms.

## Discussion

In this study, we have successfully established F344 rat prolactinoma models with MRI monitoring. After  $17\beta$ -estradiol treatment for 6 weeks, the tumor weight, tumor volume, and serum PRL level increased obviously. Our data show a decreasing trend of the DRD2 level with gradually inducing prolactinoma in F344 rat prolactinoma models. We also have demonstrated that small-animal PET with  $^{18}\text{F}$ -fallypride and  $^{18}\text{F}$ -FDG can be used to obtain nonbiased results with expression of DRD2 and evaluate the tumorigenesis of

prolactinoma in vivo. There is some potential correlation between in vitro and in vivo measurements of DRD2. We detected the level of DRD2 is lower in prolactinoma of F344 rat prolactinoma model than in normal pituitary of F344 rats by using molecular imaging technique in vivo, and the results was consistent with the detected level of DRD2 by using PCR and Western blot methods. Bodei et al<sup>15</sup> did much research about the in vivo and in vitro detection of DRD2 in uveal melanomas, and the results of in vivo scintigraphy were in line with immunohistochemical evidence of DRD2 expression in tumor samples too.

DRD2 is mainly expressed in pituitary lactotrophs where they are involved in the inhibition of PRL release and synthesis and lactotroph proliferation.<sup>16,17</sup> Furthermore, the level of DRD2 in the prolactinoma of estrogen-induced F344 rat prolactinoma models was decreasing significantly which was measured by using PCR and Western blot methods. These observations suggest a physiologically relevant molecular mechanism by which circulating sex hormones could modulate the DRD2 level in the pituitary.<sup>18,19</sup> Several dopaminergic mechanisms may contribute to the experience of action–effect associations.<sup>20</sup>

In the *Drd2*<sup>-/-</sup> mouse, the expected phenotype of chronic hyperprolactinemia and prolactinoma development was observed, and this model proved to be valuable for studying

dopamine-resistant prolactinomas where angiogenic contribute to prolactinoma development.<sup>21</sup> The treatment of prolactinoma with DAs is partly efficacious and many studies have shown that the DRD2 expression level correlates well with the clinical response to DAs treatment in prolactinomas.<sup>22</sup>

MRI revealed a focal-enhanced mass extending over the pituitary gland, but changes were observed after 4 and 6 weeks of 17 $\beta$ -estradiol inducing. PET scan was performed and showed less uptake of <sup>18</sup>F-fallypride and more uptake of <sup>18</sup>F-FDG in tumor groups compared to the control group. We found that the tumor vessels were increasing with the 17 $\beta$ -estradiol-induced prolactinoma successfully, and the uptake of <sup>18</sup>F-FDG in prolactinomas was increased, but the uptake of <sup>18</sup>F-fallypride in prolactinoma declined.

In the pituitary, <sup>18</sup>F-fallypride is an effective molecular imaging tracing agent that targets to DRD2 in pituitary diseases. PET imaging with <sup>18</sup>F-FDG, an analog of glucose, tracks the glucose metabolism of tissues. The integral role of <sup>18</sup>F-FDG-PET in oncology is indisputable.<sup>23</sup> Small-animal PET and small-animal MRI were useful in evaluating and monitoring the tumorigenesis. Ramakrishnan et al<sup>7</sup> demonstrated that spontaneous pituitary tumors in aged rats can be detected and distinguished from normal pituitary and brain using small-animal PET imaging with the  $\sigma$ -1 ligand, <sup>11</sup>C-SA4503. With the development of high-affinity radioligands such as <sup>11</sup>C-FLB457, <sup>18</sup>F-desmethoxyfallypride, <sup>18</sup>F-fallypride, and the radioligand <sup>11</sup>C-Raclopride that have been the most widely used for binding to the DRD2/dopamine receptor-D3,<sup>24</sup> it has become possible to noninvasively quantify extrastriatal DRD2/dopamine receptor-D3 densities during pharmacologic and nonpharmacologic paradigms.

Muhr et al<sup>25</sup> and Muhr and Bergstrom<sup>26</sup> performed PET studies with <sup>11</sup>C-labeled dopamine D2 antagonists such as <sup>11</sup>C-N-raclopride and <sup>11</sup>C-N-methylspiperone for the in vivo measurement of D2 receptors in prolactinomas and growth hormone-secreting pituitary adenomas. They have demonstrated higher dopamine receptor binding in patients who are responsive to DAs than in therapy-resistant patients. Abi-Dargham et al<sup>27</sup> indicated that using <sup>123</sup>I-IBZM-SPECT could provide direct evidence of increased stimulation of DRD2 by dopamine in schizophrenia, and measuring DRD2 in patients with schizophrenia could be done using radiotracers that are either sensitive <sup>11</sup>C-raclopride or insensitive <sup>11</sup>C-N-methylspiperone. The introduction of newer and more receptor-specific radioligands may expand the clinical use of these techniques in the future. The availability of PET for the clinical diagnosis of pituitary tumors is still limited, but promising results have been described.

In the field of nuclear medicine, PET techniques have become available for the study of dopamine receptors, estrogen receptors, and somatostatin receptor subtype expression in many diseases. <sup>18</sup>F-fallypride targets to D2/D3 receptors in vivo, so it could be seen widely in distribution. Not only extrastriatal, but also striatal D2/D3 receptor densities can be visualized with <sup>18</sup>F-fallypride as a radiotracer for PET studies. But there is mainly DRD2 in the pituitary and prolactinoma; this study provides direct in vivo evidence that <sup>18</sup>F-fallypride could be used to detect the level of DRD2 in normal pituitary and prolactinoma in animal experiments or preclinical tests. <sup>18</sup>F-fallypride has potential value in the assessment of patients who have DAs resistance due to low levels of DRD2 in the prolactinoma.

<sup>18</sup>F-FDG is the most commonly used radioactive tracer worldwide. When <sup>18</sup>F-FDG is administered systemically, it is accessible to essentially all tissues. As an isomer of glucose, it is usually absorbed and transported into the brain.<sup>28</sup> In F344 rat prolactinoma models, there is more uptake of <sup>18</sup>F-FDG in prolactinoma compared to the normal pituitary; it was consistent with the VEGF immunohistochemistry staining results. In the clinical applications, <sup>18</sup>F-FDG was used to detect tumors in the whole human body. Conversely, another PET tracer <sup>68</sup>Ga DOTATATE has a high affinity to somatostatin receptors, and <sup>18</sup>F-fallypride mainly has a high affinity to DRD2 in pituitary as well. Zhao et al<sup>29</sup> indicated that combination of <sup>18</sup>F-FDG and <sup>68</sup>Ga DOTATATE PET imaging is potentially valuable in the delineation between recurrent or residual pituitary adenoma and the remaining normal pituitary tissue after transsphenoidal adenectomy. From what was mentioned above, we could predict that PET with <sup>18</sup>F-fallypride and <sup>18</sup>F-FDG is potentially valuable in assessing the tumorigenesis of the prolactinoma in vivo as well. Our research will provide basic evidence to the in-depth research in rat prolactinoma models, and probably could provide evidence to clinical applications in the future.

## Conclusion

The negative modulation of DRD2 expression and the positive action on hyperprolactinemia in the serum by estrogen revealed the complex estrogen regulation in F344 rat prolactinoma models. PET with <sup>18</sup>F-fallypride and <sup>18</sup>F-FDG in assessing the tumorigenesis of the prolactinomas in vivo may be worthwhile. This method may be potentially useful for diseases associated with DRD2 and the detection of DRD2 level in prolactinoma could be an indication of chemotherapy treatment. We could predict that “noninvasive molecular imaging” like “molecular markers” will become widely and properly used in research and medicine, and benefit humanity in the future.

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## Disclosure

The authors report no conflicts of interest in this work.

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