Comparative Medicine
Copyright 2016
by the American Association for Laboratory Animal Science

Vol 66, No 1
February 2016
Pages 25–29

Original Research

Effect of Gonadectomy on the Androgen-Dependent Behavior of Ganglion Cell-Like Cells in Djungarian Hamsters (*Phodopus sungorus*)

Rei Nakahira, Rumika Yoshida,† Masaki Michishita, Kozo Ohkusu-Tsukada, and Kimimasa Takahashi*

Ganglion cell-like (GL) cells reside in the dermis of the ventral skin of mature male Djungarian hamsters (*Phodopus sugorus*) and express androgen receptor (AR). To assess whether GL cells have androgen-dependent behavior, we evaluated the histologic changes of GL cells after gonadectomy. Five male and 5 female hamsters were gonadectomized at the age of 4 wk and necropsied 14 wk later. The number, distribution, and proliferative activity of GL cells in the thoracoabdominal and dorsal skins were evaluated histologically and compared with those of corresponding intact animals. GL cells were more numerous, were distributed throughout the skin more widely, and had higher proliferative activity in the intact male hamsters than in their gonadectomized counterparts. Similar trends regarding these 3 parameters were seen in ovariectomized compared with intact female hamsters and between intact male and intact female hamsters. These results suggest that the GL cells of Djungarian hamsters demonstrate sex-associated differences in their distribution and proliferative activity and that androgen may be involved in the development of these cells.

Abbreviations: AR, androgen receptor; GL cell, ganglion cell-like cell.

Ganglion cell-like (GL) cells reside in the dermis of the abdominal and thoracic skin of mature male Djungarian hamsters (*Phodopus sungorus*).[1,7] GL cells have a small round nucleus with an apparent nucleolus and abundant basophilic foamy cytoplasm. These cells usually aggregate to form nests accompanied by various volumes of stromal collagen fibers. The nests increase in size and number with maturation. The nuclei of GL cells have a positive reaction for androgen receptor (AR), and the cytoplasm is positive for vimentin.[1,7] Although the morphologic characteristics and various immunophenotypes of GL cells have been documented, their behavior and role remain almost unclear. Some authors speculate that increased levels of testosterone may influence GL cell proliferation and the oncogenesis of atypical skin fibromas preferentially arising in this species.[1]

The current study aims to elucidate the androgen-dependent behavior of GL cells and compare the histologic changes of GL cells in gonadectomized Djungarian hamsters with those in intact control animals.

Materials and Methods

Animals. Male and female Djungarian hamsters (age, 4 wk; Saitama Experimental Supply, Sugito, Japan) were kept in aluminum press cages (22 cm × 32 cm × 11 cm; Tokiwa Kagaku Kikai, Tokyo, Japan) containing corncob bedding (Green-Tru, Green Products, Conrad, Iowa) under a controlled environment maintained at 22 ± 1.5 °C, with 50% ± 20% relative humidity and 12:12-h light:dark cycles and had unrestricted access to pelleted food (NMF, Oriental Yeast, Tokyo, Japan) and tap water. Hamsters were housed as 1 pair per cage for mating. All pups were kept under similar conditions. Breeding was repeated as needed to obtain the number of animals necessary for this experiment. Serologic monitoring against specific pathogens was performed every 6 mo by using Djungarian hamsters housed in the same animal room as the study population. The most recent results confirmed the lack of serum antibodies against *Clostridium piliforme*, *Mycoplasma pulmonis*, Sendai virus, and mouse hepatitis virus. Hamsters were also free of *Pseudomonas aeruginosa*, *Salmonella* spp., *Pasteurella pneumotropica*, *Citrobacter rodentium*, *Corynebacterium kutscheri*, *Mycoplasma* spp., dermatophytes, *Giardia* spp., *Spironucleus muris*, and *Syphacia* spp.

The study hamsters (n = 10 per sex) were allocated into a gonadectomized group (5 male, 5 female) and an intact group (5 male, 5 females); 2 or 3 animals from the same group and sex were housed together. For gonadectomy, the animals were injected with pentobarbital sodium (32.4 mg/kg IP; Kyoritsu Seiyaku, Ltd., Tokyo, Japan) and anesthetized, the testes and ovaries were removed aseptically, and the skin incisions were swabbed with 80% ethanol after suturing.

All animals were euthanized by pentobarbital overdose at 18 wk of age, and the thoracoabdominal and dorsal skins were collected. All experimental procedures were approved by the Ani-

Received: 28 Apr 2015. Revision requested: 07 Jun 2015. Accepted: 05 Aug 2015.
Department of Veterinary Pathology, School of Veterinary Medicine, Nippon Veterinary and Life Science University, Tokyo, Japan,
†Current affiliation: Laboratory of Theriogenology, University of Miyazaki, Miyazaki, Japan
**Corresponding author.Email: kimimasa@nvlu.ac.jp*

mal Experiments Committee of the Nippon Veterinary and Life Science University (no. 26K-30).

Histologic evaluation and scoring of number and distribution of GL cells. Thoracoabdominal and dorsal skins were stretched slightly, pinned on a small piece of cardboard, fixed in neutral-buffered 10% formalin, and crosscut craniocaudally into 16 strips, each of which was 4 to 6 mm in width. Strips were numbered in craniocaudal order. In addition, the testes and ovaries of intact male and female hamsters were sampled and fixed. For routine histopathologic examination, the formalin-fixed skin strips and gonads were embedded in paraffin wax. Paraffin sections were cut (thickness, 4 µm) and stained with hematoxylin and eosin.

For each strip of thoracoabdominal or dorsal skin, the number of GL cells was graded as follows: grade 0, no GL cells found; grade 1, small isolated nests of GL cells present; and grade 2: large fused nests of GL cells present (Figure 1). The grades for all strips of the same number were summed by group and presented as a ratio relative to the total grade.

Measurement of nest thickness of GL cells. For each animal, the 3 largest GL nests were photographed at a magnification of 200×. The maximal vertical thickness of the nests was measured by using Photoshop CS3 software (Adobe Systems, San Jose, CA).

Evaluation of proliferative activity of GL cells. For evaluation of proliferative activity of GL cells, serial parafinized sections were immunostained by using an antibody to proliferating cell nuclear antigen (PCNA; dilution, 1:200; clone PC10, DAKO, Glostrup, Denmark) as the primary antibody and an immunoperoxidase polymer reagent (EnVision+/HRP, DAKO). Heat-induced antigen retrieval (121 °C for 10 min in citrate buffer, pH 6.0) was performed as a pretreatment procedure. Nonspecific binding was blocked by incubating sections for 1 h at room temperature in 2% Block Ace Powder (DS Pharma Biomedical, Osaka, Japan) in PBS (pH 7.2). Sections then were incubated overnight at 4 °C by using the primary antibody and in the polymer reagent for 30 min at room temperature. The reaction was visualized by adding diaminobenzidine tetrahydrochloride chromogen and counterstaining with hematoxylin. To confirm the specificity of PCNA immunostaining, the reaction of the nuclei of epidermal basal cells was used as an internal positive control. The specificity of the reaction was confirmed by replacing the primary antibody with normal mouse serum. The nests of GL cells in immunostained sections were randomly photographed at 400-fold magnification. All of the GL cells and PCNA-positive GL cells in 5 fields were counted, and the PCNA-positive index (%) was expressed as the number of PCNA-positive GL cells / the total number of GL cells × 100%.

Statistical analysis. The Statcel 3 add-in (OMS Publishing, Saitama, Japan) for Microsoft Excel was used for statistical analysis. For grading the number of GL cells, an individual average was calculated from the grades of all skin strips; a group average was calculated from these individual averages; and group averages were compared statistically by Kruskal–Wallis and Steel–Dwass testing. For the remaining parameters, group averages were calculated from individual values and compared by using Kruskal–Wallis and Steel–Dwass testing. Differences were considered statistically significant at a P of less than 0.05.

Results

Histologic evaluation of growth of GL cells. GL cells were found in all groups of hamsters regardless of sex, although they differed in number and distribution between groups. The population of GL cells was concentrated at the middle zone of the ventral skin. In intact male hamsters, GL cells were most numerous and showed the widest distribution: they occurred throughout the ventral skin as well as in some dorsal skin strips (Figure 2). In comparison, intact female and castrated male hamsters had considerably fewer GL cells, which were restricted to the ventral skin (as for ovariectomized females) than did intact males. The GL cell grade (mean ± 1 SD) of intact male hamsters (1.83 ± 0.15) was significantly ($P < 0.05$)

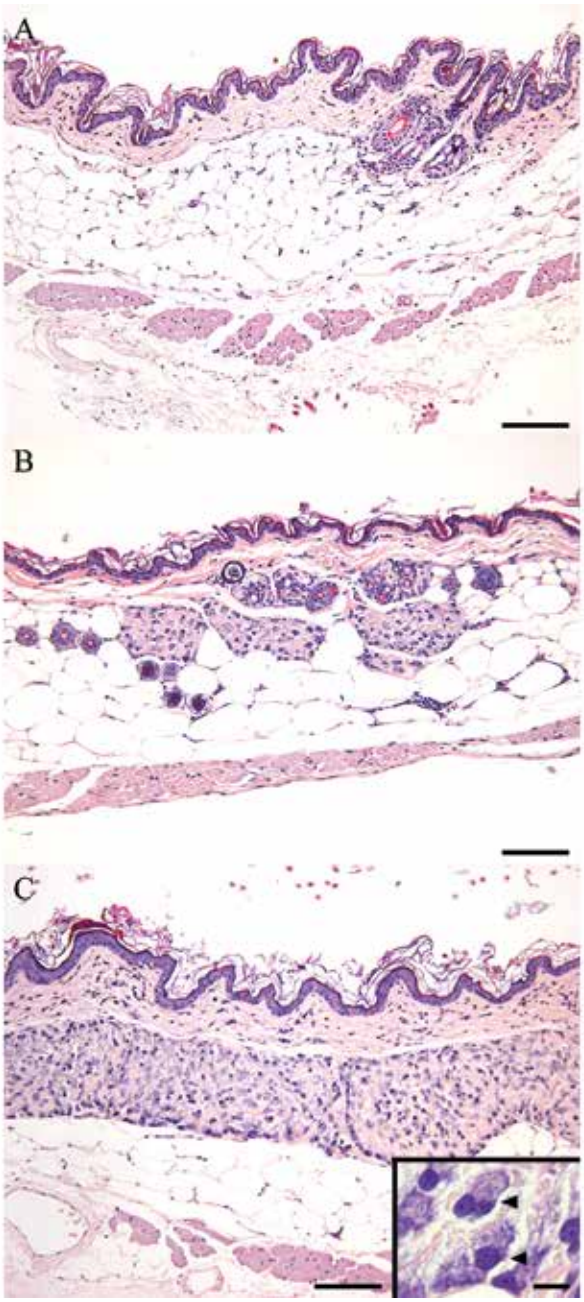

Figure 1. Growth of GL cells. Grade 0: no GL cells present. Bar, 100 µm. (A). Grade 1: small isolated nests of GL cells are present. Bar, 100 µm. (B). Grade 2: GL cells are readily found as large, fused nests in the dermis to subcutaneous tissue. Bar, 100 µm. (C) GL cells (arrowheads) have round nuclei and basophilic foamy cytoplasm. Bar, 10 µm (inset). Hematoxylin and eosin stain.

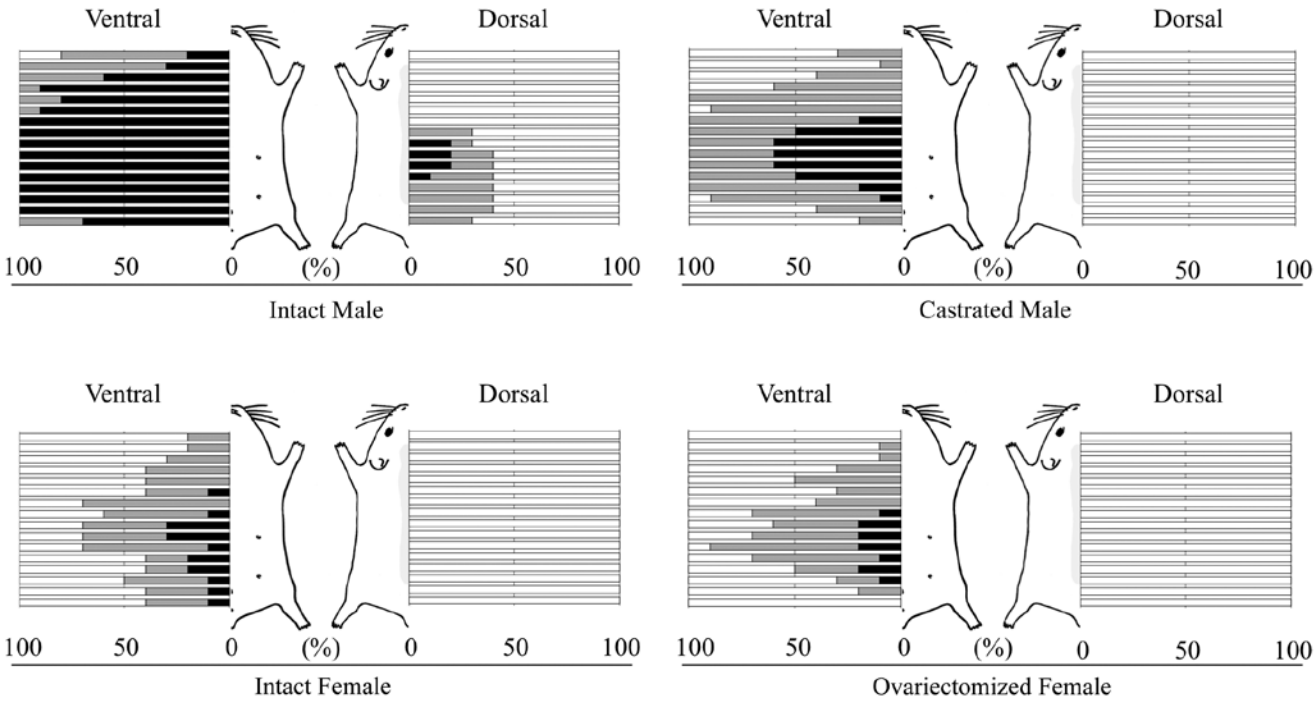

Figure 2. Average rate (%) and grade of GL cell nests in each group. Each bar represents the ratio of the GL cells of the indicated grade to the total number of GL cells in the skin strip. Grade 0 (white): no GL cells found; grade 1 (gray): GL cells present as small isolated nests; and grade 2 (black): GL cells occur as large, fused nests.

higher than that of castrated males (0.94 ± 0.27) or intact females (0.56 ± 0.56). In contrast, the average GL cell grade did not differ between intact and ovariectomized female hamsters.

Thickness of GL cell nests. GL cell nests were thicker ($P < 0.05$; Kruskal–Wallis and Steel–Dwass tests) in the intact male hamsters (236.79 ± 111.71 μm) than in castrated male hamsters (122.37 ± 54.21 μm). GL cell nest thickness did not differ between the ovariectomized (93.52 ± 69.15 μm) and intact (94.07 ± 42.11 μm) female hamsters.

Proliferative activity of GL cells. The PCNA index of GL cells was significantly ($P < 0.05$) higher for the intact male hamsters (86.85 ± 3.75%) than that for the castrated males (74.08% ± 9.31%), intact females (81.36% ± 4.63%), or ovariectomized females (67.30% ± 10.71%; Figure 3). In addition, the intact female hamsters showed a significantly ($P < 0.05$) higher PCNA index, compared with the gonadectomized males and females. The proliferative rate of GL cells in the intact male or female hamsters was greater ($P < 0.05$) than that of their gonadectomized counterparts.

Discussion

In intact male hamsters, the nests of GL cells developed widely throughout the thoracoabdominal ventral skin and often expanded into the dorsal skin. In comparison, GL cells were less prevalent in the thoracoabdominal ventral skin and were completely absent from the dorsal skin of gonadectomized male and female hamsters and intact females. These current results coincide with the distribution of the GL cells described previously.[1,7] In addition, the pattern of the frequent atypical skin fibromas in this species corresponds highly with the distribution of GL cells.[1] Moreover, these tumor cells express AR and are morphologically similar to GL cells.[1] These features suggest that these tumors may derive from GL cells. In addition to the marked sex-associated difference in GL cell distribution, the PCNA index of the GL cells and thus their proliferative capacity was higher in the intact male hamsters than in the intact females. A similar trend in the PCNA index of GL cells has been reported previously.[7]

In the castrated male hamsters, the GL cell number, distribution, nest size, and PCNA index were clearly lower than those in the intact males. Therefore, gonadectomy decreased the proliferative activity of GL cells, leading to their restricted distribution. The primary hormonal change induced by male gonadectomy is thought to be a decrease in serum androgen concentration in humans[12] and several other species.[4] Most of the biologic effects of androgens are mediated by AR, which activates downstream androgen-dependent signaling pathway networks and ultimately exerts its transcriptional effects by binding to DNA sequences termed 'androgen response elements' that are associated with androgen-regulated genes.[11] Androgenic stimulation through AR induces cell proliferation in the male reproductive system and is implicated in the development and progression of prostate cancer,[10] bladder cancer,[9] and a subtype of breast cancer.[5] Therefore, the poorly developed GL cell nests in the castrated male hamsters may have been caused by a lack of androgen–AR signaling.

The PCNA index of the GL cells in ovariectomized female hamsters was decreased compared with that in intact females, although neither their number nor distribution differed between these groups. Ovariectomy is known to significantly decrease the androgen concentration in postmenopausal women.[2,8] Similarly, the ovary in Djungarian hamsters may influence their serum testosterone level. The fact that the proliferative activity of GL cells was decreased in gonadectomized animals of both sexes compared with their intact counterparts could be explained by low serum levels of androgen due to gonadectomy.

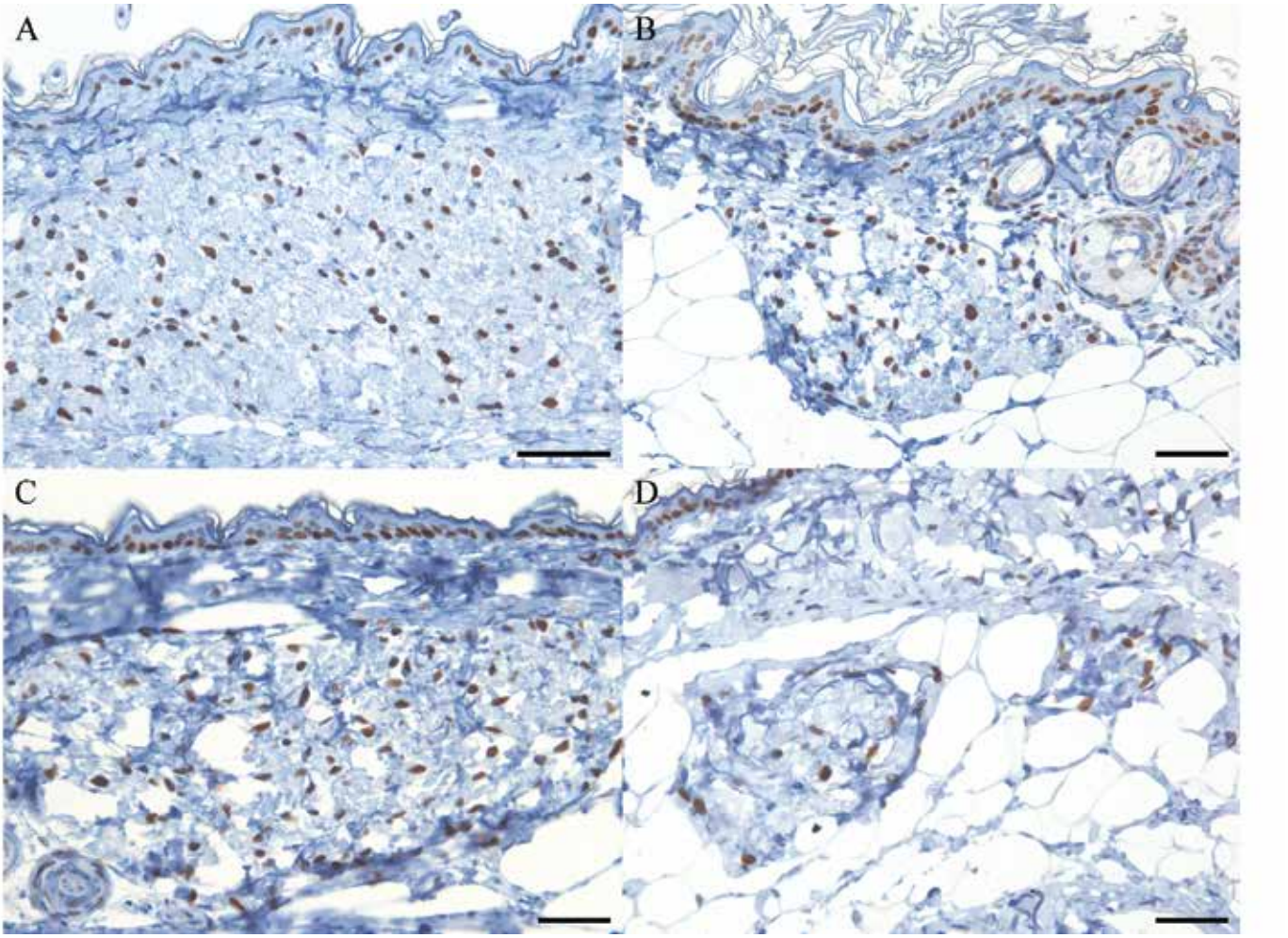

Figure 3. PCNA immunoreactivity of GL cells. Except for those of ovariectomized female hamsters, GL cells were highly positive for PCNA. (A) Intact male hamsters. (B) Castrated male group. (C) Intact female hamsters. (D) Ovariectomized female hamsters. Hematoxylin counterstain; bar, 50 µm.

The physiologic significance of GL cells remains unknown. The Harderian glands of Syrian hamsters[6] and the abdominal scent glands in gerbils[3,13] are thought be sexually dimorphic and to produce a pheromone associated with interindividual communication. However, unlike those glandular cells, GL cells are mesenchymal cells, and it is unlikely that they produce pheromones. The role of the GL cells in male Djungarian hamsters may be quite unique.

In conclusion, the GL cells of Djungarian hamsters have clear sex-associated differences in their distribution and proliferative activity, both of which were limited by gonadectomy. In addition to their previously described AR expression, the development of GL cells likely is influenced by androgens such as testosterone.

Acknowledgment

We thank Ms. Junko Sano for her excellent illustrations.

References

1. **Baba Y, Takahashi K, Nakamura S.** 2003. Androgen-dependent atypical fibromas spontaneously arising in the skin of Djungarian hamsters (*Phodopus sungorus*). Comp Med **53:**527–531.
2. **Bui HN, Struys EA, Martens F, de Ronde W, Thienpont LM, Kenemans P, Verhoeven MO, Jakobs C, Dijstelbloem HM, Blankenstein MA.** 2010. Serum testosterone levels measured by isotope dilution–liquid chromatography–tandem mass spectrometry in postmenopausal women versus those in women who underwent bilateral oophorectomy. Ann Clin Biochem **47:**248–252.
3. **Deutschland M, Denk D, Skerritt G, Hetzel U.** 2011. Surgical excision and morphological evaluation of altered abdominal scent glands in Mongolian gerbils (*Meriones unguiculatus*). Vet Rec **169:**636–641.
4. **Fukuda S, Iida H.** 2000. Effects of orchidectomy on bone metabolism in beagle dogs. J Vet Med Sci **62:**69–73.
5. **Garay JP, Karakas B, Abukhdeir AM, Cosgrove DP, Gustin JP, Higgins MJ, Konishi H, Konishi Y, Lauring J, Mohseni M, Wang GM, Jelovac D, Weeraratna A, Sherman Baust CA, Morin PJ, Toubaji A, Meeker A, De Marzo AM, Lewis G, Subhawong A, Argani P, Park BH.** 2012. The growth response to androgen receptor signaling in ERα-negative human breast cells is dependent on p21 and mediated by MAPK activation. Breast Cancer Res **14.1:**R27.
6. **García-Macia M, Rubio-Gonzalez A, de Luxán-Delgado B, Potes Y, Rodríguez-González S, de Gonzalo-Calvo D, Boga JA, Coto-Montes A.** 2013. Autophagic and proteolytic processes in the Harderian gland are modulated during the estrous cycle. Histochem Cell Biol **141:**519–529.

7. **Kashida Y, Ishikawa K, Arai K, Mitsumori K.** 2003. Morphological characterization of skin ganglion-like cells in Djungarian hamsters (*Phodopus sungorus*). Vet Pathol **40:**548–555.
8. **Kulak J Jr, Urbanetz AA, Kulak CA, Borba VZ, Boguszewski CL.** 2009. Serum androgen concentrations and bone mineral density in postmenopausal ovariectomized and nonovariectomized women. Arq Bras Endocrinol Metabol **53:**1033–1039.[Article in Portuguese]
9. **Li Y, Izumi K, Miyamoto H.** 2012. The role of the androgen receptor in the development and progression of bladder cancer. Jpn J Clin Oncol **42:**569–577.
10. **Massie CE, Lynch A, Ramos-Montoya A, Boren J, Stark R, Fazli L, Warren A, Scott H, Madhu B, Sharma N, Bon H, Zecchini V, Smith DM, Denicola GM, Mathews N, Osborne M, Hadfield J, Macarthur S, Adryan B, Lyons SK, Brindle KM, Griffiths J, Gleave ME, Rennie PS, Neal DE, Mills IG.** 2011. The androgen receptor fuels prostate cancer by regulating central metabolism and biosynthesis. EMBO J **30:**2719–2733.
11. **Matsumoto T, Sakari M, Okada M, Yokoyama A, Takahashi S, Kouzmenko A, Kato S.** 2013. The androgen receptor in health and disease. Annu Rev Physiol **75:**201–224.
12. **Michiel Sedelaar JP, Dalrymple SS, Isaacs JT.** 2013. Of mice and men—warning: intact versus castrated adult male mice as xenograft hosts are equivalent to hypogonadal versus abiraterone- treated aging human males, respectively. Prostate **73:**1316–1325.
13. **Thiessen DD, Friend HC, Lindzey G.** 1968. Androgen control of territorial marking in the Mongolian gerbil. Science **160:** 432–434.