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Myxomatous neoplasms in the perineal region of baboons

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Abstract

Background—In baboons, *Papio* sp. neoplasms tend to affect the hematopoietic system most commonly, with rare documentation of myxomatous neoplasms. In contrast, women can develop myxomatous masses within deep peripelvic tissues with some frequency during their reproductive years.

Methods—We have identified and examined, retrospectively, myxomatous perineal masses in twelve female baboons within one research facility and compared their histopathologic, immunohistochemical and electron microscopic features to their human variants.

Results—Our results indicate that these myxomatous neoplasms, in humans and non-human primates, share common features.

Conclusion—Further research, particularly molecular genetic analysis, may be needed to identify the baboon as a true animal model for myxomatous perineal neoplasms.

Keywords

Aggressive angiomyxoma; angiomyofibroblastoma; cdk4; estrogen receptor; MDM2; progesterone receptor

Introduction

Myxomas and myxosarcomas are mesenchymal tumors of fibroblast origin composed of stellate to spindle shaped cells separated by an abundant mucinous stroma. These neoplasms are generally rare in domestic and exotic animals. A literature search identified reported myxomas in the horse [2, 22], cow [19, 20, 24], dog [4, 10, 30], bird [32], snake [3], hamster [27], cat [8], and sheep [26]. Tumor locations included the nasal cavity, heart, spleen, subcutis, synovium and joints, spinal canal, and respiratory tract.

In humans, there are various myxomatous to fibrous neoplasms that can occur in the perivulvar, peripelvic, and perineal regions. These neoplasms include aggressive angiomyxoma, angiomyofibroblastoma. Superficial angiomyxoma, myxoid malignant

fibrous histiocytoma and dermatofibrosarcoma protuberans are myxofibromatous neoplasms that occur in varied dermal and subcutaneous sites.

A recent review of spontaneous neoplasia in male and female baboons, *Papio* sp., reported the hematopoietic system to be the most commonly affected organ system, with lymphoma the most commonly diagnosed neoplasm [9]. Myxomas are considered rare occurrences in non-human primates with one case in the appendicular skeleton [28] and two in the peripelvic region reported. Martino et al. [18] described well demarcated, expansive, tan to red, firm masses within the perineal region of two female baboons. These masses were composed of spindled myxoid-type cells, with little atypia and a low mitotic rate; the tumors were diagnosed as low-grade spindle sarcomas with features of aggressive angiomyxoma. Within this report, Martino et al. also proposed an association between these myxomas, other identified neoplasms and chronic *Herpesvirus papio* infection [18].

Non-human primates in research provide a foundation for monitoring spontaneous neoplasms in colonies over several years. Monitoring, identifying, classifying and relating genetics and reproductive cycle/productiveness to this particular neoplasm in baboons could provide valuable information relative to its human neoplastic counterpart. Such collected data may provide further insight to the biological behavior of human angiomyxomas and an avenue to improved treatment options.

This report describes a retrospective study of gross, histologic, immunohistochemical and ultrastructural features of a myxomatous neoplasm identified predominantly in the perineal region of 12 female baboons at one research facility. To our knowledge, there are no previous reports that classify or characterize this neoplasm in the baboon and its relationship to its human counterparts.

Materials and methods

Perineal masses or swellings were identified in 12 female baboons from the Southwest National Primate Research Center, San Antonio, TX. Baboons ranged in age from 14 to 25 years and samples of the masses were obtained as biopsy specimens, during necropsy after euthanasia or natural death from various causes. This lesion has yet to be reported in male baboons at this facility.

Additionally, normal skin from the perineal region of five female baboons was used for comparison to the affected animals. Samples were obtained from two- and three-week-old infants and from five-, nine- and sixteen-year-old adults.

For histologic evaluation, formalin fixed tissues were paraffin embedded, sectioned, stained with hematoxylin and eosin and coverslipped routinely.

All immunohistochemical procedures were performed on unstained sections of formalin fixed, paraffin embedded tissue after routine deparaffinization (Table 4). Staining of both MDM2 and cdk4 used the DAKO Universal Plus Autostainer with biotin-free signal amplification (Catalyzed Signal Amplification II DAKO, Carpinteria, CA) for visualization. Rabbit polyclonal antibody for MDM2 (LabVision, Fremont, CA) was used in a dilution of 1:50 with background reducing antibody diluent (DAKO, Carpinteria, CA) and incubated overnight at 4°C. Antigen retrieval for MDM2 was performed using Citra (Biogenex, San Ramon, CA) in a pressure cooker (Biocare Medical) at 125°C for 30 minutes. The chromagen used was 3,3'-diaminobenzidine (DAB) (DAKO, Carpinteria, CA). The sections were counterstained with hematoxylin (DAKO, Carpinteria, CA) and then cover-slipped. Mouse monoclonal antibody for cdk4 (Biosource, Camarillo, CA) was used in a dilution of 1:50 with background reducing antibody diluent (DAKO, Carpinteria, CA) and incubated

overnight at four degrees C. Antigen retrieval for cdk4 was performed using Trilogy (Cell Marque, Hot Springs, AR) in a pressure cooker (Biocare Medical) at 125°C for 30 minutes. The chromagen, counterstain and cover slip procedures were identical to MDM2. Mouse monoclonal antibodies for CD34, estrogen receptor (ER) and progesterone receptor (PR) were employed at ready to use dilutions (Ventana Medical Systems, Tucson, Arizona). Mouse monoclonal antibody for Des 33 (DAKO, Carpinteria, CA) was used at a 1:200 dilution and melan A (Vector Laboratories, Burlingame, CA) was used at 1:50 dilution. Antigen retrieval on the preceding five antibodies was performed using CC1 cell conditioning (Ventana Medical Systems, Tucson, Arizona) for 30 minutes followed by application of the primary antibody for 32 minutes on the Ventana Benchmark Autostainer (Tucson, AZ). The immunohistochemical signal was visualized with the basic DAB detection kit (Ventana Medical Systems, Tucson, AZ) and counterstained with hematoxylin (Ventana Medical Systems, Tucson, AZ). Rabbit polyclonal antibody for S100 (DAKO, Carpinteria, CA) was used at a 1:600 dilution with an application of 24 minutes. Mouse monoclonal antibody for alpha smooth muscle actin (SIGMA, St. Louis, MO), was used at a dilution of 1:1600 with an application time of 32 minutes.

A representative sample was chosen for ultrastructural examination. A formalin-fixed sample of perineal skin from case 10 was post-fixed in 1% osmium tetroxide, dehydrated and embedded in Effapoxy (Ernest F. Fullam, Latham, New York), sectioned at 80 nm, stained with 4% uranyl acetate and Reynolds lead citrate. Sections were examined and photographed on a Zeiss EM 10 electron microscope (LED Electron Microscopy Inc., Thornwood, New York).

Vascular density scoring was based on the number of blood vessels evident per 20× field utilizing H&E sections and on vascular wall smooth muscle actin and Des33 immunohistochemical immunoreactivity. This assessment was deemed necessary to identify broad variations and to further classify these tumors compared to their human counterpart. Tissues were scored as (1) high vascular density (>10 vessels/20×) (2) moderate vascular density (6–10 vessels/20×) or (3) low vascular density (2–5 vessels/20×). The matrix was scored as (a) abundant matrix (0–20 neoplastic cells/hpf), (b) moderate matrix (21–45 neoplastic cells/hpf) or (c) scant matrix (>46 neoplastic cells/hpf).

Gross and histologic findings

The neoplasms in this study were subcutaneous and ranged from 6 cm to 20 cm in diameter and were generally found in the perineal region. Varied sites, one tumor each, included caudal thigh, left hip, surface of the abdomen and vagina (Table 1). On cut section, masses were firm, white or dark, gelatinous and glistening (Fig. 1). Grossly, they were poorly circumscribed and blended with the surrounding normal appearing tissue.

Histologically, neoplasms were unencapsulated, poorly circumscribed, and expanded by an abundant vascular and myxomatous matrix. Neoplastic cells were spindle to stellate, with variably distinct borders and scant, eosinophilic fibrillar cytoplasm. Nuclei were elongate with finely stippled chromatin and one variably distinct nucleolus (Fig. 2). No mitoses were observed. Neoplastic cells were generally uniform and occasionally binucleate. Blood vessel morphology varied between cases. The majority of cases demonstrated variably sized thin-walled vessels scattered throughout a myxomatous matrix. Thick-walled vessels surrounded by thin smooth muscle bundles that radiated into the surrounding tissue were prominent in case 9 (Fig. 3). Mixed throughout the neoplasm were few to moderate lymphocytes, few mast cells, plasma cells and neutrophils, often surrounding blood vessels. Fifty-eight percent (7/12) of the neoplasms demonstrated pigmented stellate cells scattered throughout the dermis. Two cases (cases 4 and 6) demonstrated few to moderate neutrophils. Case number

6 demonstrated a moderate neutrophilic infiltrate with an overlying ulcer and moderate granulation tissue.

Vascular and myxomatous density varied throughout neoplasms. Matrix and vascular density scoring for each case are listed in Table 2; see materials and methods for determination of scoring matrix. The majority of our cases demonstrated a moderate to high vascular density with a moderate myxomatous matrix.

Of the specimens used as controls, four demonstrated normal collagen arrangement with no spindle cell or myxomatous expansion. Surprisingly, one control, from a sixteen-year-old female that had been used for breeding (case 13), demonstrated similar spindle to stellate cells widely expanded by a myxomatous matrix as the described neoplasms, but with no reported mass effect.

Immunohistochemical findings

Immunohistochemical findings are noted in Table 3 and Figs 4–8. Similar to previously published work, a tissue sample was considered positive for MDM2 or cdk4 by light microscopy if at least one cell nucleus per high powered field stained with the antibody [6]. The majority of neoplasms were immunoreactive for estrogen and progesterone receptors, smooth muscle actin, Des33, MDM2 and cdk4 and non-reactive for S100 and CD34. Stellate dendritic cells demonstrated multifocal reactivity for S100 and mel A. Mel A was performed only on case 9 to confirm that cytoplasmic granules within dendritic cells were melanin. Case 13, submitted as a control of normal baboon skin with similar histologic features as the myxomatous lesions, demonstrated immunoreactivity for estrogen and progesterone receptors, multifocal immunoreactivity for Des 33 and smooth muscle actin and was similarly non-reactive for CD34 and S100. This case demonstrated non-specific staining for MDM2, and cdk4 was unsuccessful.

Ultrastructural findings

Ultrastructural examination revealed spindle to stellate cells with oval to rarely reniform nuclei and occasionally prominent nucleoli (Fig. 9). Cells contained moderate to abundant rough endoplasmic reticulum with dilated cisternae (Fig. 10). Suboptimal tissue preservation precluded finer examination of cytoplasmic contents. Cells were widely dispersed in a low-density myxoid matrix with few collagen fibrils (Fig. 10).

Discussion

In humans, the myxomatous to fibrous neoplasms that occur in the perivulvar, peripelvic, and perineal regions include aggressive angiomyxoma, angiomyofibroblastoma, and cellular angiofibroma. Myxofibromatous neoplasms occurring in dermal and subcutaneous sites include superficial angiomyxoma, myxoid malignant fibrous histiocytoma and dermatofibrosarcoma protuberans. In humans, infiltrative aggressive angiomyxomas occur in the deep pelviperineal soft tissue, can be difficult to excise, often recur locally and rarely metastasize [7]. Women in their third to fifth decades are most often affected, with rare reported cases in pre-pubertal or post-menopausal females [21]. These tumors occasionally present, in males, in the scrotum or perineum.

Aggressive angiomyxoma is a locally invasive tumor, in humans, that develops within the pelviperineal soft tissue. It consists of fibroblasts, myofibroblasts and thick-walled vessels often surrounded by thin streams of smooth muscle separated by an abundant myxoid matrix and may differentiate toward specialized genital stroma [21]. These tumors are immunoreactive for vimentin, smooth muscle actin and desmin with some authors reporting

positivity for estrogen and progesterone receptors and general non-reactivity for S100 protein [1, 11, 14, 25, 33]. Our baboon cases similarly demonstrated 100% estrogen and progesterone reactivity and the majority were Des33 and smooth muscle actin immunoreactive. Case 9 most closely resembled aggressive angiomyxoma in tumor depth, location and histologic features. This case demonstrated a moderate vascular density and moderate myxomatous matrix; blood vessels were thick-walled and often surrounded by whorls of smooth muscle.

Angiomyofibroblastoma is an important differential diagnosis for myxomatous neoplasms of the perineal and vulvar regions. However, this tumor is characterized by small size, defined tumor boundaries with numerous polymorphic spindle cells that are occasionally multinucleated and giant, separated by thin walled vessels and no extravasation of erythrocytes [13, 21]. Granter et al. [14] describe plump epithelioid tumor cells arranged perivascularly as a feature distinguishing this neoplasm from aggressive angiomyxoma. All specimens examined in our group were poorly circumscribed with vessels ranging from thin to thick walled and blended with the surrounding stroma. Additionally, although not exclusionary in itself, epithelioid tumor cells were not observed.

Angiomyofibroblastomas have neoplastic stromal cells that are uniformly negative for alpha smooth muscle actin, S100, cytokeratin, myosin, myoglobin and CD68 with homogenous staining for vimentin and CD44 and multifocal desmin and muscle specific actin [5, 33]. Immunoreactivity for desmin has been said to be the differentiating factor between angiomyofibroblastoma and aggressive angiomyxoma because it can reflect myofibrillary differentiation [13]. However, author opinions vary on the importance of desmin immunoreactivity. Skalova et al. [29] posit that the immunophenotype of aggressive angiomyxoma and angiomyofibroblastoma can be identical and Amezcua et al. [1] report that immunohistochemistry is of little help because desmin immunoreactivity is also reported in aggressive angiomyxomas.

Superficial angiomyxoma is a multilobular, dermal or subcutaneous mass with poorly defined margins. Histologically it is composed of spindle to stellate cells with no cytologic atypia or pleomorphism but demonstrates scattered mitoses and a mixed inflammatory infiltrate with a prominent number of neutrophils, which is considered a useful marker. They are consistently immunoreactive for vimentin and are variably immunoreactive for CD34, muscle specific and smooth muscle actin, S100 and factor XIIIa [21]. Although few tumors in our study presented as lobular or superficial masses, (cases 4 and 11), our cases were consistently non-reactive for CD34. All cases presented with a minimal to mild perivascular mononuclear infiltrate and few cases demonstrated few to moderate numbers of neutrophils (cases 4 and 6). Case number 6 demonstrated a moderate neutrophilic infiltrate; however, there was an overlying ulcer and moderate granulation tissue.

Myxoid fibrous histiocytoma is characterized as a myxomatous tumor with varied small or bland cells and enlarged, bizarre and pleomorphic cells and prominent blood vessels. Immunohistochemically, a high percentage of cells are actin positive [15]. Kurman et al. [16] reported immunoreactivity of neoplastic cells for alpha1-antitrypsin; this immunohistochemical marker was not used in our study. Although we reported a significant percentage of cells immunoreactive for smooth muscle actin, all of our cases presented with bland, stellate cells and relatively no pleomorphism.

Myxoid dermatofibrosarcoma protuberans is a locally aggressive spindle-cell mesenchymal neoplasm that presents, in humans, as multinodular cutaneous plaques [25]. Microscopically they are not encapsulated and have low mitotic activity in myxoid areas and varied activity in more storiform regions. Immunohistochemistry is similar to aggressive angiomyxomas

with immunoreactivity for vimentin and an absence of reactivity for S100 protein. They are intensely immunoreactive for CD34, a possible differentiating marker. The lack of reactivity in our cases for CD34, a general absence of mitotic figures and a subcutaneous location preclude classification of the neoplasms in our study as dermatofibrosarcoma protuberans.

The majority of our cases (83%) presented with masses in the perineal region. Two cases (cases 3 and 12) presented with masses arising in the vagina and vulva, respectively. Vulvar lymphedema in humans has been described as a proliferation of fibroblastic cells in an edematous-myxoid stroma and vessels with perivascular collagen deposition, mimicking aggressive angiomyxoma [31]. Lymphedema was considered as a differential diagnosis for case number 12 but due to the presence of an actual mass, compared to generalized enlargement documented in humans, and the absence of ectatic lymphatics, we chose to classify that case along with the other myxomatous neoplasms.

Ultrastructural studies indicate that aggressive angiomyxomas derive from a primitive mesenchymal cell normally found in the lower female genital tract, that is capable of myofibroblastic differentiation [12]. Ultra-structural studies performed by Skalova et al. [29] and Martinez et al. [17] reported neoplastic cells with the morphology of fibroblasts, smooth muscle cells or a mixture of both. Ultrastructurally, myofibroblasts demonstrate well developed cell-stroma attachments (fibronexi), intermediate cellular junctions, fibronectin fibrils on the cell surface, well-developed endoplasmic reticulum and golgi apparatus [17, 29]. Smooth muscle cells demonstrate a basement membrane, attachment plaques and plasmalemmal caveolae [17]. Cells of myxoid dermatofibrosarcoma protuberans have sparse fibroblast-like cells with elongated nuclei with rare indentations, prominent rough endoplasmic reticulum, some cisternae-like dilatations, electron dense fine granules and short microfilaments [25]. Case 9, examined ultrastructurally in our study, demonstrated fibroblast-like features with moderate to abundant rough endoplasmic reticulum and dilated cisternae. Due to suboptimal tissue preservation, finer examination of neoplastic cells in our study was not possible. Because of this restriction, a comparison of our ultra-structural findings cannot be made to findings established in aggressive angiomyxomas. Future studies to fully characterize the ultrastructural features of these tumors in the baboon are warranted.

In humans, chromosomal rearrangements at 12q15 are frequent in many mesenchymal tumors, including aggressive angiomyxoma, uterine leiomyoma and atypical lipomatous tumors [11]. These rearrangements are clustered within a region containing the HMGIC gene, whose product is important in transcriptional regulation and is normally expressed in fetal tissue but not in normal adult tissue [23]. Genetically, these mesenchymal tumors can be characterized by amplification of MDM2 and cdk4 genes on chromosome 12q13–15 [3]. cdk4 and MDM2 are genes delineating the up- and downstream ends of 12q13–15 [11]. Targeted gene(s) affected by HMGIC expression may explain this tumor's interactive phenotype; that is HMGIC, might interact with a molecular pathway in the subepithelial mesenchymal tissue of the vulva and scrotum giving rise to aggressive angiomyxoma [23]. Binh et al. [6], while determining the immunoreactivity of atypical lipomatous tumors for cdk4 and MDM2, found cdk4 staining to be less sensitive than MDM2 staining because cdk4 is not always coamplified with the mdm2 chromosome 12q13–15 amplicons. In our study, we demonstrated 91% and 41% immunoreactivity of tumors for MDM2 and cdk4, respectively. Although these immunohistochemical markers identify many mesenchymal tumors and are not specific for aggressive angiomyxomas, our results suggest a similar genetic rearrangement in the baboon.

All tumor cases, as well as case 13, a sixteen-year-old baboon submitted as normal perianal skin, were immunoreactive for estrogen (ER) and progesterone receptors (PR). Perianal skin samples from the remaining four control baboons were negative for estrogen and

progesterone receptors. There is speculation in the human literature as to the role hormones play in these tumors, with tumors occurring almost exclusively in women of fertile age and in a selective anatomic location [15]. Aggressive angiomyxomas are arguably derived from the myxoid vascular stroma of the peri-pelvic and perineal region [15]. The myxomatous changes identified in case 13 is curious. The fact that this female was used as a breeder allows for speculation as to the role hormones play in the development of these tumors. Had case 13 lived longer, the possibility that this region could have developed into a neoplasm should be considered.

Available reproductive histories of these baboons were examined to determine if a correlation existed between tumor or myxomatous lesion formation and age of the animal, number of offspring produced and ER/PR expression. Seven of 11 baboons that developed tumors, had five or more offspring within their lifetime and ranged in age from 12 to 25-years-of-age. Four baboons that developed tumors, were nulliparous and ranged in age from 15 to 25-years-of age. Of four of five normal perianal skin controls, three animals were not of reproductive age; one control was nine years old and produced one offspring. Case 13, the remaining perianal skin control, was 16-years-old and produced the highest number of offspring (twelve) off all animals included in the study. From our small sample size of normal control tissues, it appears that ER and PR expression within perianal tissue may be dependent on age of the animal (i.e number of reproductive cycles endured), number of offspring produced and possibly the stage of reproductive cycle at time of tissue harvest. Of the two reproductively active baboons submitted as controls, these factors could explain the difference in ER and PR expression; one control tissue was immunoreactive and the other was not.

Case 13 may be an example of sex skin at a particular stage of the menstrual cycle or sex skin transitioning to a neoplastic process. A study establishing a baseline of sex skin histology and associated immunohistochemical features at each stage of the menstrual cycle of baboons of varied ages and reproductive history would be beneficial in identifying normal skin under hormonal influence. These established baseline findings, when compared to confirmed neoplasms demonstrating a mass effect, may elucidate the morphologic and immunohistochemical transition to a neoplastic process.

In summary, we have described the gross, histologic, immunohistochemical and ultrastructural characteristics of an angiomyxomatous tumor in the perianal region of the baboon. The neoplasms presented in these 12 cases had features resembling those of human aggressive angiomyxoma and angiomyofibroblastoma. Histologically and grossly, variability in location depth and variable presence of thick-walled vessels surrounded by whorls precludes exact classification of the baboon neoplasm in either category. Immunohistochemical results support findings in both human counterparts. Therefore, we propose classification of these neoplasms as angiomyxoid genital stromal tumors.

Unfortunately, the biological behavior of this newly identified neoplasm in the baboon could not be assessed in this retrospective study. Better recognition of this tumor, with excision and close clinical follow up, should facilitate elucidation of its biological behavior as compared to its proposed human counterparts. Further molecular genetic analysis for genes such as HMGI-C in fresh baboon tissue may allow further comparison and evaluation of the baboon as an animal model for human peripelvic myxomatous tumors.

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References

1. Amezcua CA, Begley SJ, Mata N, Felix C, Ballard CA. Aggressive angiomyxoma of the female genital tract: a clinicopathologic and immunohistochemical study of 12 cases. *Int J Gynecol Cancer*. 2005; 15:140–5. [PubMed: 15670309]
2. Barakzai SZ, Dixon PM. Sinonasal myxoma in a four-year-old thoroughbred gelding. *Vet Rec*. 2003; 152:210–1. [PubMed: 12620038]
3. Barten SD, Frye FL. Leiomyosarcoma and myxoma in a Texas indigo snake. *J Am Vet Med Assoc*. 1981; 179:1292–5. [PubMed: 7328025]
4. Berrocal A, Millan Y, Ordas J, de las Mulas JM. A joint myxoma in a dog. *J Comp Pathol*. 2001; 3:223–6.
5. Bigotti G, Coli A, Gasbarri A, Castagnola D, Madonna V, Bartolazzi A. Angiomyofibroblastoma and aggressive angiomyxoma two benign mesenchymal neoplasms of the female genital tract: an immunohistochemical study. *Pathol Res Pract*. 1999; 195:39–44. [PubMed: 10048093]
6. Binh MB, Sastre-Garau X, Pineux GD, Terrier P, Lagace R, Aurias A, Hostein I, Coindre JM. MDM2 and CDK4 immunostainings are useful adjuncts in diagnosing well-differentiated and dedifferentiated liposarcoma subtypes: a comparative analysis of 559 soft tissue neoplasms with genetic data. *Am J Pathol*. 2005; 29:1340–7.
7. Blandamura S, Crus J, Vergara LF, Puerto IM, Ninfo V. Aggressive angiomyxoma: a second case of metastasis with patient's death. *Hum Pathol*. 2003; 34:1072–4. [PubMed: 14608546]
8. Campbell MD, Gelberg HB. Endocardial myxoma of the right atrium of a cat. *Vet Pathol*. 2000; 37:460–2. [PubMed: 11055869]
9. Cianciola RE, Gubbard GB. A review of spontaneous neoplasia in baboons (*Papio spp*). *J Med Primatol*. 2005; 34:51–66. [PubMed: 15860111]
10. Darke PG, Gordon LR. Cardiac myxoma in a dog. *Vet Rec*. 1974; 95:545–7.
11. Graadt van Roggen JF, van Unnik JAM, Briare-de Bruijn IH, Hogendoorn PCW. Aggressive angiomyxoma: a clinicopathological and immunohistochemical study of 11 cases with long-term follow up. *Virch Arch*. 2005; 446:157–63.
12. Granter SR, Nucci MR, Fletcher CDM. Aggressive angiomyxoma: reappraisal of its relationship to angiomyofibroblastoma in a series of 16 cases. *Histopath*. 1997; 30:3–10.
13. Havel G, Burian P, Kohrtz M, Mark J. Aggressive angiomyxoma of the vulva: an unusual, deceptive and recurrence-prone tumour with evidence of estrogen receptor expression. *AMNIS*. 1994; 102:236–40.
14. Kazmierczak B, Wanschura S, Meyer-Bolte K, Caselitz J, Meister P, Bartnitzke S, Van de Ven W, Bullerdiek J. Cytogenetic and molecular analysis of an aggressive angiomyxoma. *Am J Pathol*. 1995; 147:580–5.
15. Kempson, RL.; Fletcher, CDM.; Evans, HC.; Hendrickson, MR.; Sibley, RK. Malignant fibrous histiocytoma. In: Rosai, editor. *Atlas of Tumor Pathology, Tumors of Soft Tissues*. Washington, DC: AFIP; 1990. p. 166-73.
16. Kurman, RJ.; Norris, HG.; Wilkinson, E. Tumors of the cervix, vagina and vulva. In: Rosai, editor. *Atlas of Tumor Pathology*. Washington, DC: AFIP; 1990. p. 166-73.
17. Martinez MA, Ballestin C, Carabias E, Lois CG. Aggressive angiomyxoma: an ultrastructural study of four cases. *Ultrastruct Pathol*. 2003; 27:227–33. [PubMed: 12907367]
18. Martino MA, Hubbard GB, Butler TM, Hilliard JK. Clinical disease associated with simian agent 8 infection in the baboon. *Lab Ani Sci*. 1998; 48:18–22.
19. Misra SS, Angelo SJ, Azad CS. Interdigital myxoma in a Haryana cow. *Vet Med Small Anim Clin*. 1981; 76:381. [PubMed: 6908984]

20. Mohiyuddin S, Malaki A. A case of primary myxoma of subcutaneous region in a cow. *Indian Vet J.* 1967; 44:613–5. [PubMed: 6070768]
21. Murphy, GF.; Elder, DE. Fibrohistiocytic tumors. In: Rosai, editor. *Atlas of Tumor Pathology, Non-Melanocytic Tumors of the Skin.* Washington, DC: AFIP; 1990. p. 227-8.p. 428-32.
22. Murphy JR, Breeze RG, McPherson EA. Myxoma of the equine respiratory tract. *Mod Vet Pract.* 1978; 59:529–32. [PubMed: 672887]
23. Nucci MR, Weremowica S, Neskey DM, Sornberger K, Tallini G, Morton CC, Quade BJ. Chromosomal translocation t(8;12) induces aberrant HMGIC expression in aggressive angiomyxoma of the vulva. *Genes Chromosomes Cancer.* 2001; 32:172–6. [PubMed: 11550285]
24. Opsomer G, De Bosschere H, Vanroose G, Hoflack G, De Vlieghe S, de Kruif A. Aggressive angiomyxoma in a cow. *Vet Rec.* 2001; 149:594–5. [PubMed: 11730170]
25. Orlandi A, Bianchi L, Spagnoli LG. Myxoid dermatofibrosarcoma protuberans: morphological, ultrastructural and immunohistochemical features. *J Cutan Pathol.* 1998; 25:386–93. [PubMed: 9765025]
26. Paliwal OP, Baxi MR. Pulmonary myxoma in sheep. *Indian Vet J.* 1972; 56:892. [PubMed: 536043]
27. Ridgway RL. Spontaneous myxoma in a pet hamster. *Vet Med Small Anim Clin.* 1977; 72:75. [PubMed: 584102]
28. Shalev M, Murphy JC, Fox JG, Wallstrom AC, Gottlieb LS. Myxoma of bone in a nonhuman primate. *Cancer.* 1980; 45:2573–82. [PubMed: 6769581]
29. Skalova A, Michal M, Husek K, Zamecnik M, Leivo I. Aggressive angiomyxoma of the pelvioperineal region. *Am J Dermatopathol.* 1993; 15:446–51. [PubMed: 8238780]
30. Teague HD, Berg JA. Myxoma of the spinal canal in a dog. *J Am Vet Med Assoc.* 1978; 173:985–6. [PubMed: 721681]
31. Vang R, Connelly JH, Hammill HA, Shannon RL. Vulvar hypertrophy with lymphedema: a mimic of aggressive angiomyxoma. *Arch Pathol Lab Med.* 2000; 124:1697–9. [PubMed: 11079029]
32. West JL. An avian splenic myxoma. *Avian Dis.* 1974; 18:139–41. [PubMed: 4814552]
33. White J, Chan Y-F. Aggressive angiomyxoma of the vulva in an 11-year old girl. *Pediatric Pathol.* 1994; 14:27–37.

Fig. 1.
Perineum, gelatinous mass from case six.

Fig. 2.
Perineum, case seven. A moderate number of variably sized blood vessels are separated by a moderate myxomatous matrix. Hematoxylin and eosin. (2x)

Fig. 3.
Perineum, case nine. A denser component of thick walled blood vessels are demonstrated.
Hematoxylin and eosin. (4x)

Fig. 4. Perineum, case seven. Stellate to spindled neoplasm cells are immunoreactive for smooth muscle actin. Smooth muscle actin. (20×)

Fig. 5.
Perineum, case nine. Neoplastic cells demonstrate strong intranuclear immunoreactivity for estrogen receptor. Estrogen receptor. (20×)

Fig. 6. Perineum, case nine. Neoplastic cells demonstrate strong intranuclear immunoreactivity for progesterone receptor. Progesterone receptor. (20×)

Fig. 7.
Perineum, case seven. Neoplastic cells demonstrate strong intracytoplasmic immunoreactivity for MDM 2. MDM 2. (40×)

Fig. 8. Perineum, case ten. Neoplastic cells demonstrate intracytoplasmic reactivity for cdk4. There is mild background staining. Cdk4. (40×)

Fig. 9.
Electron microscopy, case ten. A spindled neoplastic cell, separated by a moderate myxomatous stroma with few collagen bundles. Collagen bundles are indicated by arrow. (3150 $\times$)

Fig. 10.
Electron microscopy, case ten. A stellate neoplastic cell with abundant endoplasmic reticulum and dilated cisternae. Dilated cisternae are indicated by arrow. (10 000 $\times$)

Table 1

General gross and clinical findings

Case	Age (years)	Sex	Mass location	Size	Other histologic and gross findings
1	19	F	Surface of abdomen	1	Multiple ovarian cysts
2	22	F	Bilateral, raised, within perineum	15 cm	Ovarian, pituitary, parathyroid and uterine cysts; hyperplasia of oviducts
3	19	F	Epithelial covered mass protruding from vagina	4 × 8 cm	Peritonitis; pancreatitis; gastric edema; cholecystitis
4	18	F	Dark mass, top of left hip	1	Cellulitis; chronic, active epidermal hyperplasia
5	16	F	Perineum	4 cm × 8 cm	Multiple ovarian cysts
6	17	F	Perineum	6 cm × 6 cm	³
7	25	F	Perineum	1	Lung cysts; uterine adenomyosis, endometriosis; spondylosis
8	17	F	NI	1	Hydronephrosis; spondylosis
9	14	F	Deep, dark, gelatinous perineum	1	Melanosis of perineum amyloidosis, islets of Langerhans; colitis
10	17	F	Perineum	10 × 30 cm	Uterine endometriosis and adenomyosis
11	20	F	Right caudal thigh	2 cm	³
12	12	F	Vulva	1	Histoplasmosis, sex skin
13	16	F	Normal perianal skin	2	

¹Size of neoplasm not indicated in clinical history.

²Normal skin submitted as control, no mass lesion was identified.

³Information not provided.

Table 2**Histologic grading**

High vascular density/abundant matrix (1a)	1/12 (8.3%)
High vascular density/moderate matrix (1b)	3/12 (25%)
High vascular density/scant matrix (1c)	0/12 (0%)
Moderate vascular density/abundant matrix (2a)	1/12 (8.3%)
Moderate vascular density/moderate matrix (2b)	3/12 (25%)
Moderate vascular density/scant matrix (2c)	0/12 (0%)
Low vascular density/abundant matrix (3a)	2/12 (16.6%)
Low vascular density/moderate matrix (3b)	2/12 (16.6%)
Low vascular density/scant matrix (3c)	0/12 (0%)

Table 3

Immunohistochemical findings of submitted neoplasms

CD34	0/11 (0%) ¹
S100	1/12 (8.3%)
Smooth muscle actin	10/12 (83.3%)
DES 33	8/12 (66.6%)
MDM2	11/12 (91.6%)
cdk4	5/12 (41.6%)
Estrogen receptor	12/12 (100%)
Progesterone receptor	12/12 (100%)
Mel A	0/1 (0%) ²

¹CD34 not performed on case 12.

²Only performed on case 9.

Table 4

Details of immunolabeling reagents

Primary antibody	Fixative	Antibody dilution	Pre-treatment
CD34 ¹ , monoclonal	10% buffered formalin	Ready to use	CC1 ⁴
Desmin ¹ , monoclonal	10% buffered formalin	1:200	CC1 ⁴
Estrogen receptor ¹ , monoclonal	10% buffered formalin	Ready to use	CC1 ⁴
Progesterone receptor ¹ , monoclonal	10% buffered formalin	Ready to use	CC1 ⁴
S100 ³	10% buffered formalin	1:600	None
Smooth muscle actin ⁷ , monoclonal	10% buffered formalin	1:1600	None
MeIA ² , monoclonal	10% buffered formalin	1:50	CC1 ⁴
Cdk4 ³ , monoclonal	10% buffered formalin	1:50	Trilogy ^{®5}
Mdm2 ³ , polyclonal	10% buffered formalin	1:50	Citra ^{®6}

¹Ventana Laboratories, Tucson, AZ.²Vector Laboratories, Burlingame, CA.³DAKO, Carpinteria, CA.⁴Cell Conditioning Solution (high pH).⁵Cell Marque, Hot Springs, AR.⁶Biogenex, San Ramon, CA.⁷Sigma, St. Louis, MO.