Surgical, histological, and immunohistochemical approach to spontaneous malignant mammary tumor in a fancy rat (*Rattus norvegicus domestica*): a case report

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Abstract

A domestic rat (*Rattus norvegicus domestica*) was presented to the Department of Veterinary Medicine of the University of Bari “Aldo Moro” (Bari, Italy) for the presence of a progressive growing unilateral mass. The mass was completely surgically removed, and then histologically and immuno-histochemically evaluated. The lesion was diagnosed as mammary ductal carcinoma based on histopathological examination. Immunohistochemistry staining for estrogen receptor alpha (ERα) and proliferation marker Ki-67 were found to be positive. The rat was rechecked 3 months thereafter, with total body radiographs taken for metastatic assessment, and no macroscopic evidence of tumor recurrence was observed at the surgical site. Thus, the most effective weapon remains prevention, so owners should be advised to timely spay their pets to reduce the probability of mammary tumor appearance.

Keywords Domestic rat · Mammary tumor · Surgery · Histopathology · Immunohistochemistry

Introduction

Mammary tumors are the most commonly reported form of neoplasm in companion rats (*Rattus norvegicus domestica*) (Keeble and Meredith 2009; Hocker et al. 2017). Available studies report that up to 16% of old rats develop mammary tumors, mostly related to fibroadenomas (Percy and Barthold 2007; Quesenberry and Carpenter 2012). Fibroadenomas are benign tumors that usually have well-defined borders (Keeble and Meredith 2009). The distribution of mammary tissue is extensive, and the tumors can occur anywhere from the neck to the inguinal region. Moreover, tumors can become very large and develop in both males and females (Quesenberry et al. 2004). On the other hand, mammary carcinomas can be locally invasive and metastasize (Keeble and Meredith 2009; Quesenberry and Carpenter 2012). Malignant mammary tumors can reach considerable dimensions in female rats and sometimes also in male rats (Keeble and Meredith 2009).

Early detection and surgical excision can lead to the best prognosis for fibroadenomas. However, surgical excision of mammary carcinomas could be challenging, and the tumors are prone to recurrence (Hotchkiss 1995; Keeble and Meredith 2009; Quesenberry and Carpenter 2012).

In this case report, the authors report the surgical procedure and the histological and immunohistochemical evaluation of a spontaneous malignant mammary tumor in a domestic female rat.

Case description and discussion

A 4-year-old female domestic rat (*Rattus norvegicus domestica*) was presented at the Veterinary Hospital of the Department of Veterinary Medicine—University of Bari “Aldo Moro”—Italy for the presence of a progressive growing unilateral mass (Fig. 1). The owner referred the rat was not eating and was lethargic; during the visit, it appeared emaciated and depressed. The tumor mass was located on the right side of mammary inguinal glands, and it grew rapidly in 2 months. The mass appeared firm and non-ulcerated, and manual palpation elicited pain response. Fine needle aspirate cytology was compatible with mammary malignant neoplasia: lesion displayed clusters and sheets of atypical epithelial cells with altered nucleus and large prominent nucleoli.
So, given the emergency of the clinical case, the surgical team opted for elective mastectomy without performing hematological and serum biochemical analysis, by the signature of the owner’s informed consent.

The fancy rat underwent premedication with buprenorphine (Buprenodale®, Dechra Veterinary Products s.r.l., Turin, Italy) at 0.1 mg/kg im, dexdetomidine (Dexdomitor®, Vetoquinol, Italia, Bertinoro, Italy) at 1 mg/kg. Zolazepam/tiletamine (Zoletil®, Virbac Italia, Milan, Italy) at 0.025 ml/kg was used to induce the total anesthesia. With the anesthetic effect obtained, a venous catheter was inserted for fluid therapy and, if necessary, the necessary drugs were quickly injected. Positioning in dorsal decubitus and administration of oxygen and isofluorane (3%) through a face mask using a rotary circuit (Fig. 2). Before the start of surgery, heart rate and mean arterial pressure values were recorded on which to base the subsequent evaluation of the cardiovascular response to surgery (pre-incisional values) as well the pulse oximetry and body temperature. The shearing and aseptic preparation of the skin area affected by the surgical procedure was then performed. Once the patient was stabilized in dorsal decubitus, a sterile surgical drape was placed, stuck on the patient’s skin with Backhaus atraumatic clamps. The skin was then incised with a rounded scalpel. A diamond incision was made around the breast mass. After removing the skin with Metzenbaum scissors and clamping the vessels with atraumatic mosquito forceps, we proceeded with the neckline of the subcutis and the cut of the affected perimammary musculature. The exeresis of the mass was thus carried out (Fig. 3), and the muscular planes were stitched up with 3–0 Vicryl absorbable thread, starting from the muscular and fascia planes, then under the skin and finally the skin. Throughout the procedure, measures were put in place to minimize heat dispersion, mainly ensured by positioning the patient on an air heated mat. At the end of the procedure, Meloxicam (Metacam® Boehringer Ingelheim, Italy) administered subcutaneously...
at a dose of 0.2 mg/kg and adequate surveillance/assistance ensured until the righting reflex reappeared.

Rat was hospitalized for 24 h and received fluid therapy (saline solution) and antimicrobial treatment with enrofloxacin (Baytril® injectable solution 5%, Bayer, Milan, Italy) (5 mg/kg (2.3 mg/lb) subcutaneously (SC), q 24 h). The owner was instructed to continue enrofloxacin administration for 9 days after discharge from the hospital. Meloxicam [0.5 mg/kg (0.23 mg/lb), per os, 3 days] was prescribed for analgesia. After 10 days, the stitches on the skin were removed.

The mammary tumor was sent to the Unit of Pathology of the Department of Veterinary Medicine of University of Bari Aldo Moro (Italy) for pathological analysis. The mass sized 5 × 4 × 3 cm and at a gross observation showed a nodular configuration (Fig. 3), hard on palpation, circumscribed by a capsule partially infiltrated with gray-white cutting surface (Fig. 4). For histopathology, samples of tissue were collected and fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax using an automatic tissue processor. Serial 5-µm sections from all specimens were cut with a 2030 Biocut microtome (Reichert-Jung, Germany) and mounted on glass slides (Super-Frost, Menzel-Gläser, Braunschweig, Germany). The sections were then stained with Haematoxylin and Eosin (H&E) following standard procedure and examined under a light microscope (DM4000 Leica). Digital photos were taken with OLYMPUS-DP12 camera for detection of histopathological alterations.

Histologically, the tumor mass presented lobules with glandular differentiation consisting of solid sheets of epithelial cells with little or no visible acinar structure remaining. Tumor epithelial cells, pleomorphic in appearance, with a marked variation in cell size and shape, had scarce cytoplasm, vesicular nucleus, and single or multiple prominent nucleoli, modest the presence of mitotic figures (Fig. 5). The expanded tumor mass infiltrated the muscle underlying the mammary gland. The tumor was classified according with Russo et al. (1989) in ductal carcinoma. Histological analysis of the surgical margins showed that the excision of cancer was complete.

For a prognostic evaluation, immunohistochemical staining (IHC) was used to determine the expression of estrogen receptors (ER-α) and Ki-67 proliferative index performed using a three-layer biotin-avidin-peroxidase system. A 5-µm thin serial sections from tissue sample were cut. After tissue pretreatment including steam antigen retrieval and protein block, poly-l-lysine-coated slides were incubated with Monoclonal Mouse: (a) ERα (clone SP1; Thermo Fisher Scientific, Waltham, MA, USA, dilution 1:200), (b) PR (clone PgR 636; Dako, Glostrup, Denmark, dilution 1:200), and (c) the MIB-5 antibody to Ki-67 (Dako, Glostrup, Denmark, dilution 1:100). The bound antibodies were visualized by using a biotinylated secondary antibody, avidin–biotin-peroxidase complex, and 3-amino-9-ethyl carbazole as chromogens (Dako, Glostrup, Denmark). Nuclear counterstaining was performed with Gill’s haematoxylin (Polysciences, Warrington, PA, USA); then, the sections were dehydrated with ethanol and xylene prior to mounting. Appropriate positive and negative controls were included with each IHC run. The morphologic interpretation of immunohistochemical stains was performed independently by 2 of the authors (NZ and AT). The case was considered positive for a given marker only when both observers agreed upon its specificity and

![Fig. 4 Mammary tumor sizing 5×4×3 cm and showing a nodular configuration](image1)

![Fig. 5 Sheets of pleomorphic epithelial cells, with a marked variation in cell size and shape, scarce cytoplasm, vesicular nucleus, single or multiple prominent nucleoli (hematoxylin–eosin, ×400)](image2)
distribution. The positively stained tumor cells were counted at ×400 magnification in a 2.37 mm² area. IHC staining for ERα and Ki-67 was found to be positive in epithelial cell nuclei of neoplastic glandular tissue with a degree of intensity observed in >20% of tumor nuclei (Fig. 6a, b). The Pr immunoreactivity was negative.

Rat mammary adenocarcinomas share several morphologic similarities with most common human breast carcinomas, so the rat may be considered a model for the study of human breast cancer (Ranieri et al. 2013). Comparative studies reveal the existence of estrogen receptors with identical properties in both systems (Escrich 1987). In human, carcinoma markers, such as estrogen receptor (ER) and Ki-67 proliferative index, are relevant for therapy strategy and prognosis (Perou et al. 2000; Sørlie et al. 2001; Cheang et al. 2009; Cuzick et al. 2011). Rodents, including rats, have been treated as disposable pets because of their small size and low cost. Several studies describe the characteristics of these models and their importance to the human disease to study a variety of aspects of breast cancer biology (Zizzo et al. 2019; Zeng et al. 2020).

In recent years, rodents are considered like pet animals and they are presented to the veterinarian for diagnosis and treatment; they deserve the same approach that any other domestic species receives.

In this case, a female rat, considered a member of the family, was brought to visit because of a rapidly grown mass on the right side of inguinal mammary glands. One of the pathologies that most frequently affect rats are mammary tumors, but although this area is highly developed in laboratory animals (induced tumors), little is known about spontaneous mammary tumors in pet rats. On clinical diagnosis, the various presentations are classified based on morphological and molecular examination. Prognosis is defined according to several parameters, tumor size and grade, the presence/absence of estrogen and/or progesterone receptors, and vascular or perineural tumor invasion (Parkin et al. 2005; Sutton et al. 2010).

In this case, the authors report the surgical procedure, the histological, and immunohistochemical evaluation of a spontaneous malignant mammary tumor in a domestic rat, histologically classified as ductal carcinoma according with Russo et al. (1989). Pharmacological treatment lacks proof so, surgical removal of the mass is currently the treatment of choice, even if the tumor is prone to recurrence and because complete mammary chain resection is not easily feasible in rats (Hocker et al. 2017) and due to persistent hormonal promoting factors secondary mass often develops following excision (Hotchkiss 1995; Keeble and Meredith 2009; Quesenberry and Carpenter 2012). So, it is necessary to carry out further studies to test new drugs that can stop tumor evolution. The most effective weapon remains prevention so owners should be advised to timely spay their pets to reduce the probability of mammary tumor appearance.

In our case, the rat was rechecked 3 months thereafter, with total body radiographs taken for metastatic assessment, and no macroscopic evidence of tumor recurrence was observed at the surgical site.

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