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Establishment and characterization of a canine xenograft model of inflammatory mammary carcinoma

L. Camacho [a,*], L. Peña [b], A. González Gil [a], S. Cáceres [a], L. Díez [b], J.C. Illera [a]

[a] Department of Animal Physiology, Veterinary Medicine School, Complutense University of Madrid, Madrid, Spain
[b] Department of Animal Medicine, Surgery and Pathology, Veterinary Medicine School, Complutense University of Madrid, Madrid, Spain

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ABSTRACT

Canine inflammatory mammary cancer (IMC) and human inflammatory breast cancer (IBC) are the most aggressive form of mammary/breast cancer. Both species naturally develop it, sharing epidemiological, clinical and histological characteristics. Thus, IMC has been suggested as a model to study the human disease. We have developed the first IMC xenograft model in SCID mice. Xenografts reproduced the histological features from the primary tumor, were highly aggressive and showed dermal tumor emboli, distinctive hallmarks of IMC/IBC. This model was hormone receptors positive and HER2 negative. Our findings showed that estrogens and androgens are locally produced in tissues. Factors related to tumor vascularization showed positive expression and xenografts with the highest expression of all analyzed vascular factors had the highest rate of tumor proliferation. The role of steroid hormones and the angio/lymphangiogenic properties found in this model, provide additional knowledge for future interventions in the diagnosis, treatment and prevention of the disease.

1. Introduction

Inflammatory breast carcinoma (IBC) and canine inflammatory mammary cancer (IMC) are considered the most malignant types of breast cancer in both humans and dogs (Susaneck et al., 1983; Tavassoli, 1999; Pérez-Alenza et al., 2001). Spontaneous IMC shares epidemiologic, histological, pathologic and clinical characteristics with IBC. Therefore, canine IMC has been suggested as a model to study the human disease (Nieto et al., 2000; Pérez-Alenza et al., 2001; Peña et al., 2003a). It has been observed a local steroid hormone production in canine IMC (Peña et al., 2003b; Illera et al., 2006; Sánchez-Archidona et al., 2007). Inflammatory carcinoma is particularly fast growing, highly angiogenic and angioinvasive, characteristics that contribute to its aggressiveness (Kleer et al., 2000). The massive invasion of dermal lymph vessels by neoplastic cells has been reported in canine IMC (Pérez-Alenza et al., 2001), which block lymph drainage causing the characteristic edema (Giordano and Hortobagyi, 2003).

Mouse modeling of human breast cancer is of great value in cancer research. There are two murine models of human IBC, MARY-X and WIBC-9 (Alpaugh et al., 1999; Shirakawa et al., 2001) but thus far, there is no murine model of canine IMC. Therefore, we aimed to establish a canine xenograft model of inflammatory mammary carcinoma in SCID mice and to obtain tumor samples from the xenograft model in five progressive time points of tumor development, in order to (a) study the differences in the tumor characteristics during its growth, (b) characterize the immunophenotype of both the original canine neoplasia and the xenografts, (c) analyze the concentrations of the steroid hormones estrone sulfate (SO4E1), estradiol (E2), androstenedione (A4), testosterone (T) and progesterone (P4) in serum samples and in tissue homogenates, as well as the expression in the tumoral tissue of estrogen receptor α and β (ERα and ERβ), androgen receptor (AR) and progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and an enzyme included in the steroidogenesis pathway, aromatase, and (d) study the tumor proliferation (Ki-67) as well as the angiogenic and lymphangiogenic properties of the disease analyzing vascular endothelial growth factor A and D (VEGF-A and VEGF-D), vascular endothelial growth factor receptor 3 (VEGFR-3) and ciclooxigenase-2 (COX-2) within the tumoral tissue.

2. Materials and methods

2.1. Animals and xenograft establishment

Twenty-eight female SCID mice (BALB/cJHan™Hsd-Prkdc^{SCID}, *Harlan Laboratories Models, S.L.*) were used, 24 for the xenograft model and 4 for the control group. The animals were housed in a

* Corresponding author. Address: Dpto. Fisiología (Fisiología Animal), Facultad de Veterinaria, Universidad Complutense de Madrid, Avda. Puerta de Hierro s/n, 28040 Madrid, Spain. Tel./fax: +34 913943860.
E-mail addresses: laura_mond@hotmail.com, jcillera@vet.ucm.es (L. Camacho).

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flexible-film isolator (Isotec, *Harlan Laboratories Models*, *S.L.*) in cages (1–2 animals per cage) measuring 28 × 12 × 12 cm, in a room with controlled environmental conditions (20–22 °C; 50–55% relative humidity; 10–15 air changes per hour; and a 12:12-h light:dark cycle). Food and water, previously sterilized, were provided*ad libitum*. All experimental procedures were performed between 10:00 and 13:00 h.

The xenograft was established directly from a 11 year old German shepherd bitch. Clinical diagnosis of IMC was based on features previously described in dogs (Susaneck et al., 1983; Pérez Alenza et al., 2001). IMC was clinically suspected in the subject with rapidly growing disease in the mammary glands and overlaying skin, characterized by diffuse involvement of multiple glands, firmness, warmth, edema, erythema, thickening, and signs of pain. The sample was collected immediately after the necropsy at the Veterinary Teaching Hospital of the Alfonso X el Sabio University. Fragments of 5 × 2 mm tissue were placed in MEM liquid with Earle's Salts with L-glutamine with Penicillin/Streptomycin [100×] (p*AA Laboratories*) and were subcutaneously implanted into the ventral side of 3 female SCID mice. Animals were previously anesthetized with isoflurane (IsoVet, *B Braun VetCare SA*, Barcelona, Spain) at 4% for induction and 1.5% for maintaining sedation, supplied in a fresh gas flow rate of 0.5 L of oxygen/minute. Other fragments of the canine tissue were fixed in neutral formalin and paraffin-embedded for histopathology and immunohistochemistry studies. The sample was histologically diagnosed as a tubulo-papillary IMC (Fig. 1(1a)) on HE-stained sections following the recent histological classification of canine mammary tumors (Goldschmidt et al., 2011). Clinical IMC was histologically confirmed when numerous neoplastic emboli in superficial dermal lymphatic vessels were observed.

When the tumors developing in mice reached approximately 2 cm in diameter they were twice successively transplanted into three SCID mice, in order to verify that the xenograft model was stable and tumors did not change histologically along the three consecutive passages. Tumors from the third passage mice were transplanted into 15 mice that were sacrificed in groups of three mice in five consecutive time points of tumor development (4, 6, 8, 10 and 12 weeks). Hence, 24 mice were finally transplanted: 9 to establish the xenograft in three successive passages and 15 to obtain the different timing samples. Tumor samples were collected, measured and weighed, and blood and multiple organs from each individual were also harvested. Studies of growth rate, animal-associated characteristics, pattern of metastasis, tumor histology, hormone levels and immunohistochemistry were conducted on the model.

The experimental protocols were approved by the Institutional Animal Care and Use Committee of the Veterinary Faculty of Madrid at Universidad Complutense Madrid, Spain. All procedures were completed in accordance with the Guide for the Care and Use of Laboratory Animals and conformed to the relevant EU Directive.

2.2. DNA profiling

One mg from xenograft samples was used to extract DNA, following a standard phenol–chloroform procedure (Sambrook et al., 1989). The obtained DNA was subjected to amplification of a sequence encoding the ribosomal RNA 12S fraction of

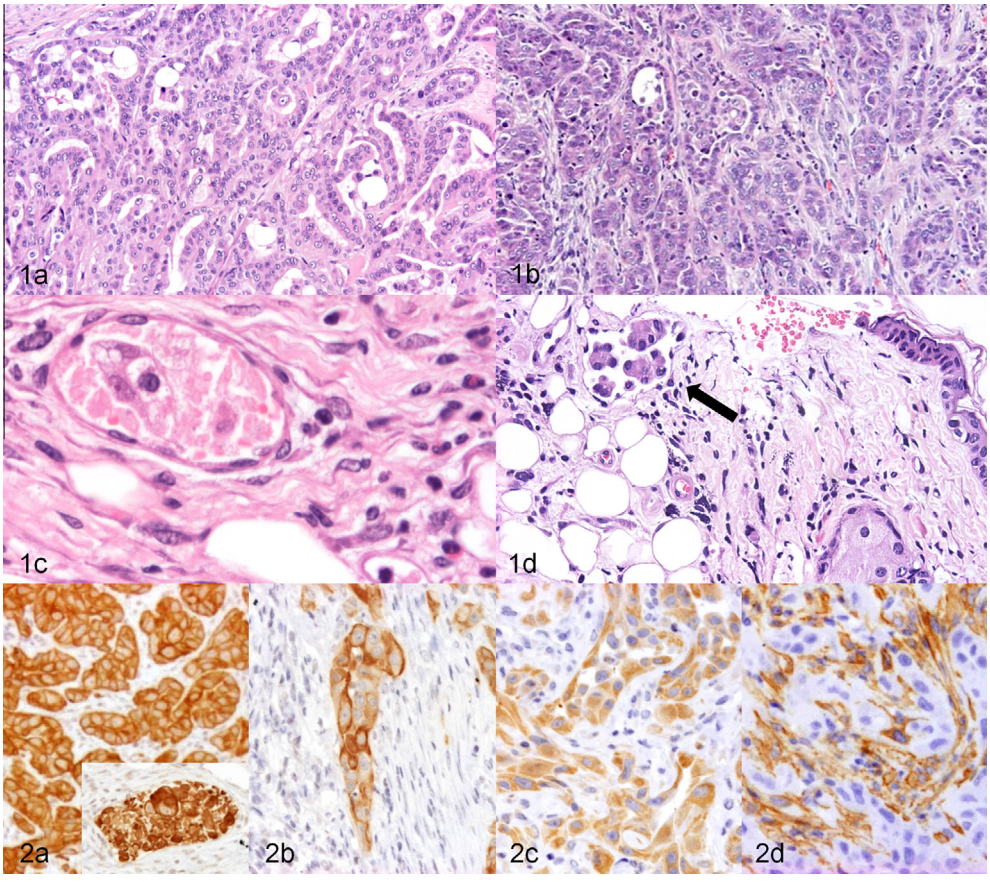

Fig. 1. (1) Inflammatory mammary carcinoma. HE. (1a). Female dog. Tubulo-papillary IMC (×10). (1b) Xenograft. Tubular solid IMC. (×10). (1c) Xenograft. Vasculogenic mimicry (×40). (1d) Xenograft. Tumor emboli in dermis (×20). (2) IMC; xenografts. Immunohistochemical characterization of tumor phenotype. Streptavidin biotin peroxidase. (2a). Anti-pancytokeratin (×20). Insert shows necrotic tumor cells in an embolus (×20). (2b). Anti-cytokeratin 14 (×20). (2c). Anti-vimentin (×20). (2d). Anti-actin (×20).