

Canine Mammary Osteosarcomas

Eva Hellmen*

Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden

Abstract

This article describes five representative cases of canine mammary osteosarcomas and induced tumours by a cloned canine mammary osteosarcoma cell line in nude mice. All five primary tumours were combined mammary osteosarcomas i.e. composed of both cartilage and bone tissues. In four of the five cases the metastases were also combined osteosarcomas. However, for the metastases in one dog and in the nude mice, only bone forming and spindle cell tumours were seen. The metastases were spread directly to the lungs by the blood in three dogs, and via the lymph nodes in two dogs. The metastases differed in morphology, both within and between different metastatic sites. Some of the metastases had an even lower grade than the corresponding primary tumour.

Keywords: Tumours; Mammary; Osteosarcomas

Introduction

Spontaneous mammary tumours frequently appear in women, dogs, cats and rodents. Their origin is known only in mice, where the mouse mammary tumour virus (MMTV) gives rise to them. Sexual hormones are involved in the development of mammary tumours; in women, oestrogen is important and most studied whereas progesterone is more important in the dog, where spaying at early age prevents mammary tumours [1]. About 50% of canine mammary tumours are benign. Benign mixed tumour, composed of epithelial cells and cartilage and/or bone tissue, is a common species specific type [2]. Malignant canine mammary tumours are dominated by carcinomas, which originate from epithelial cells. Sarcomas and mixed tumours (carcinosarcomas) also appear, and the cellular origin of the tumour types is unknown [2]. The reported incidence of canine mammary sarcomas (i.e. fibrosarcomas and osteosarcomas) ranges between 3.5% [3] and 8.3% [4]. However, canine mammary osteosarcomas represented only 1% of a large series of 10 345 mammary tumours [5]. The prognosis of canine mammary sarcomas is very poor, and about 75% of them cause metastases [4,6]. In addition to the dog, sarcomas appear in the human breast. They are however less frequent, and are often diagnosed as metaplastic carcinomas or matrix producing breast carcinomas since most are positive for epithelial markers and they often behave like carcinomas e.g. by causing metastases to the lymph nodes [7,8].

Studying metastatic canine mammary osteosarcomas can, to a certain extent, lead to a better understanding of the biology of these tumours. This article describes five representative cases of canine mammary osteosarcomas and induced tumours in nude mice. The aims of the study were to (i) describe the metastatic routes and the morphology of the metastases by careful post-mortem examination (ii) study the *in vivo* behaviour and morphology of the tumours formed in an experimental model of a cloned canine mammary osteosarcoma cell line.

Material and Methods

Primary tumours and post-mortem examination

The primary tumours were collected during surgical treatment at the former Department of Surgery and Medicine, and the dogs were post-mortem examined at the former Department of Pathology at SLU, Uppsala, Sweden. The autopsies included histological examination of most lymph nodes i.e. axillary, superficial cervical, sternal, cranial

mediastinal, tracheobronchial, popliteal, superficial and deep inguinal lymph nodes. Tissue samples were also taken and examined from all lung lobes, heart muscle, liver and kidneys, adrenals and from the ovaries when possible. The brain and skeleton were examined histologically when clinical symptoms suggested that these sites were affected. The vertebral column was bisected longitudinally and examined macroscopically, and any suspected areas were decalcified and submitted for histological examination. An x-ray of the skeleton was included in the autopsy of dog No. 353. All dogs have been included in previous publications: Dog No. O389 [9]; dog No. 117 [4]; dogs No. 143 and No. 144 [4,9,10] and dog No. 353 [10].

Histological examination

The tumours were fixed in 4% or 10% phosphate buffered formaldehyde and embedded in paraffin. The sections were cut at 4-5µm and stained with haematoxylin and eosin (HE). Osteosarcomas were classified according to the WHO classification [2]. In accordance with this classification, osteosarcomas that formed both cartilage and bone matrix were named combined osteosarcomas. However, this classification, as well as others [11], does not include grading of the sarcomas. Thus, in the present study the osteosarcomas were classified into two groups: low-grade malignancy (well differentiated and moderately differentiated) and high-grade malignancy (poorly differentiated), and grading was based on cell pleomorphism, mitotic index and matrix formation [12].

Experimental study

The primary mammary osteosarcoma in dog No. 353 gave rise to cell line CMT-U353 B, which was cloned [13]. The five clones 1, 2, 3, 6 and 7 were chosen and 5×10^6 cells were subcutaneously inoculated into each of five nude mice. The tumours that formed were fixed and stained as described for the primary tumours.

*Corresponding author: Eva Hellmen, Department of Anatomy, Physiology and Biochemistry, Swedish University of Agricultural Sciences, Uppsala, Sweden, Tel: +46 18 672128; Fax: +46-18672-111; E-mail: eva.hellmen@slu.se

Received January 09, 2013; Accepted February 28, 2014; Published March 03, 2014

Citation: Hellmen E (2014) Canine Mammary Osteosarcomas. *J Veterinar Sci Technol* 5: 163. doi:[10.4172/2157-7579.1000163](https://doi.org/10.4172/2157-7579.1000163)

Copyright: © 2014 Hellmen E. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Results

Dog No. O389 was autopsied without any previous mastectomy. A mammary combined osteosarcoma in the left (L) side caudal glands 4 to 5 was 10 x 5 x 5 cm in size. The central parts were low-grade with a large amount of bone matrix and a high-grade, more cellular periphery. Another part of the primary tumour contained both cartilage and bone tissue. Tumour invasion, with bone matrix formation even within the intravascular metastasis was seen (Figure 1A-C). Cartilage and bone also appeared in the multiple lung metastases, which varied from 1 to 4 cm in diameter. Abundant bone matrix was also seen in the lung vessel metastases (Table 1 and Figure 1D-F). In addition, several benign mammary tumours were found in the right (R) side glands 3, 4 and 5, and in L 3.

Dog No. 117 was surgically treated for a malignant mixed mammary tumour 9 cm in diameter located in gland R 3 (Figure 2A-D). Follow-up one year post-surgery, including x-ray of the lungs, showed no signs of recurrence. Two years post-surgery the dog was in good health according to the owner. The dog was euthanized and autopsied 3.5 years post-surgery. At this stage two new mammary tumours were present in

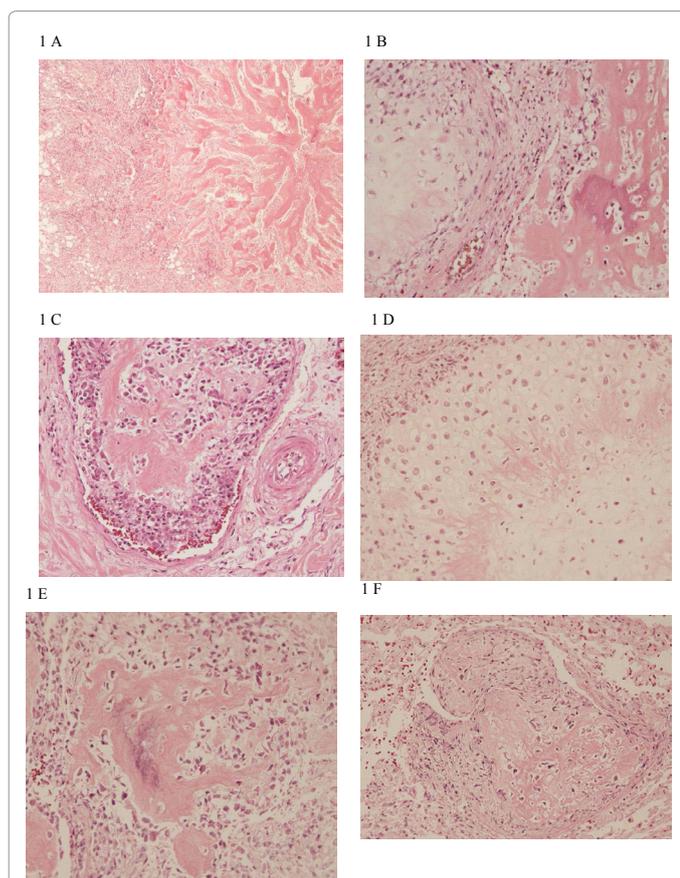


Figure 1: Primary mammary combined osteosarcoma with low-grade central parts (to the right), showing a large amount of bone matrix and a high-grade more cellular periphery (to the left), (A), (objective x 4). Another part of the primary tumour demonstrated presence of cartilage to the left and bone to the right (B), (objective x 20). Tumour invasion with bone matrix formation even within the intravascular metastasis was seen (C), (objective x 20). Cartilage (D) and bone (E) also appeared in the lung metastases (objectives x 20). Abundance of bone matrix was also seen in the lung vessel metastases (F), (objective x 20). Light micrographs of dog No. O389 with haematoxylin and eosin (HE) staining.

ID	Dog breed	Age	Primary	Metastases at post-mortem	
			mammary tumour	examination	
O 389	Boxer	Not known	Combined Osteosarcoma (L5) ^{*)}	The lungs	
117	Giant Schnauzer	11 years	Spindle cell tumour (R4) ^{*)} Combined osteosarcoma (R5)	The lungs	
143	Airedale terrier	11 years	Combined osteosarcoma (L5)	The mediastinal Inn ^{**) , lungs, myocardium and kidneys}	
144	Giant Schnauzer	11 years	Combined osteosarcoma (L5)	The sternal Inn, lungs, pericardium, myocardium, pleura, diaphragm, spleen and kidneys	
353	Papillion	15 years	Combined osteosarcoma (R1)	The lungs and kidneys	

^{*)} Tumour location: L = left side and R = right side, numbered from the cranial to the caudal mammary glands (1-5); ^{**)} Lnn = lymph nodes.

Table 1: Data of the dogs and tumours included in the study.

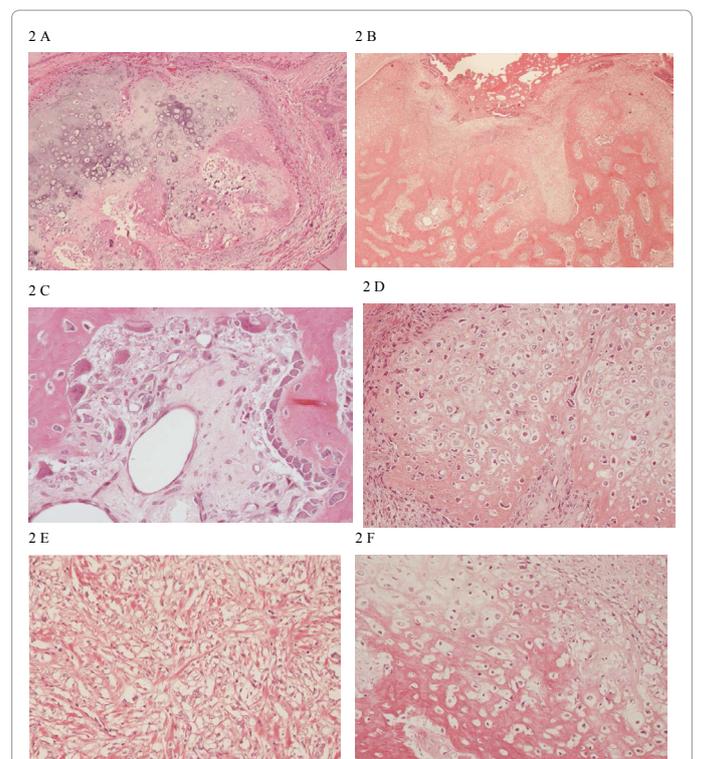
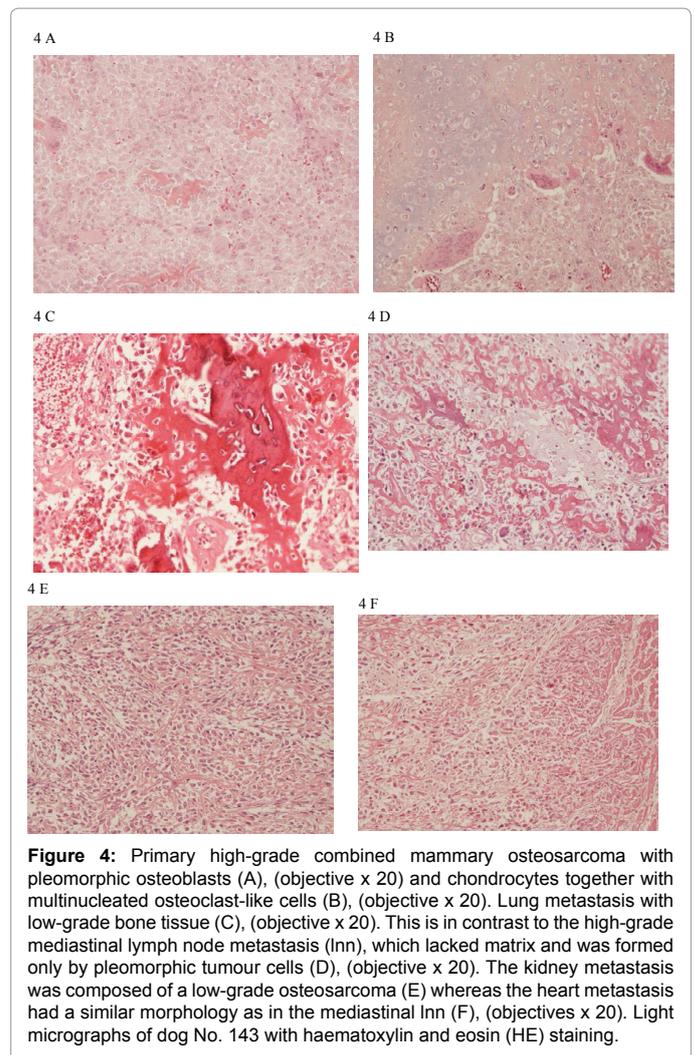
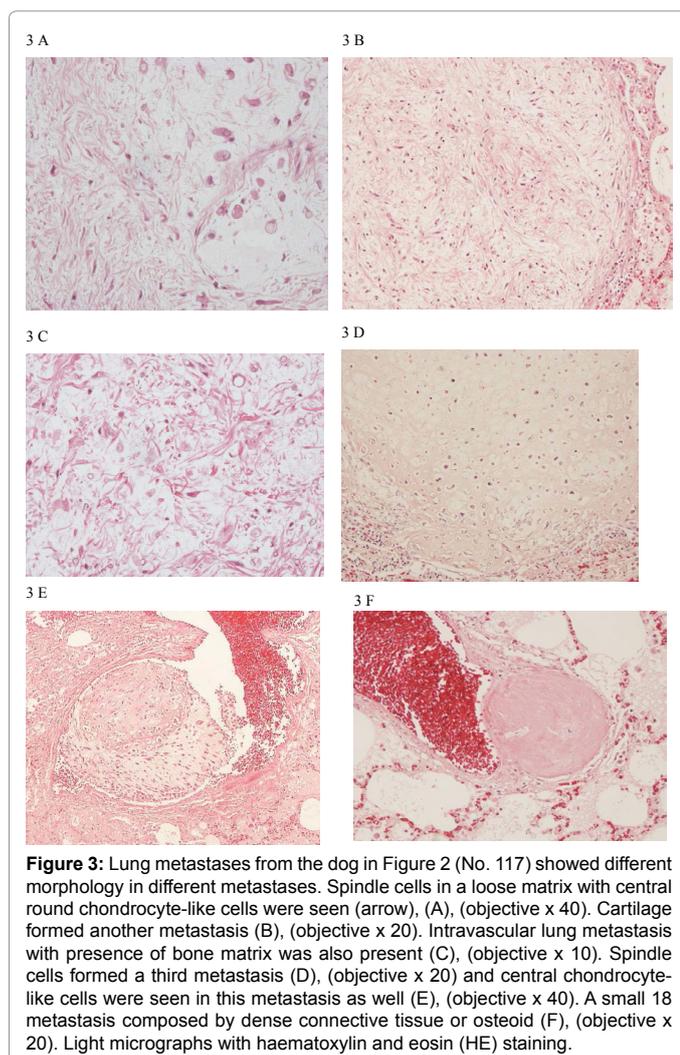


Figure 2: Primary malignant mixed mammary tumour demonstrating cartilage (A), (objective x 10) and bone tissue (B), (objective x 4). Low-grade bone tissue with osteoblasts lining the matrix to the right (arrow) and multinucleated osteoclasts to the left (*), (C) as well as less differentiated cartilage with single chondrocytes in the lacunas in the matrix (D), (objectives x 40 and x 20). Two years after surgical removal of this primary tumour (in gland R3) the dog was post-mortem examined and a spindle cell tumour was found in gland R4 (E) as well as a combined osteosarcoma in gland R5 (F), (objectives x 20). Light micrographs from dog No. 117 with haematoxylin and eosin (HE) staining.

R 4 and R 5 and were diagnosed as spindle cell tumour and combined osteosarcoma respectively (Table 1 and Figures 2E and F). The lung metastases differed in morphology; some were composed by a loose matrix with central chondrocyte-like cells (Figures 3A-C), another was formed by cartilage (Figure 3D), bone tissue in a lung vessel metastasis (Figure 3E) and a further metastasis consisted of dense connective tissue or osteoid (Figure 3F). The metastases were low-grade and very few mitoses were seen.

Dog No. 143 was surgically treated for a 7 x 6 x 4 cm mammary tumour located in gland L 5 that was diagnosed as a high grade combined osteosarcoma (Figures 4A and B). One year and 11 months post-surgery the dog was autopsied and metastases in several organs were found (Table 1). The lung metastases had a lower grade than the primary tumour (Figure 4C) whereas the kidney metastasis demonstrated less bone matrix (Figure 4D). This was in contrast to the metastases in the mediastinal lymph nodes and myocardium, which were high grade (Figures 4E and F).

Dog No. 144 was surgically treated for a mammary tumour 14 cm in diameter located in gland L 5 that was diagnosed as a combined osteosarcoma (Figure 5A). Only a minor focal area showed presence of chondroid cells (Figure 5B). Three months post-surgery, the dog was autopsied and metastases in several organs were found (Table 1). The



lung metastases were low grade compared to the primary osteosarcoma, with abundant presence of bone matrix (Figures 5C and D). The diaphragm metastases were high grade and composed of pleomorphic tumour cells that infiltrated the skeletal muscle cells (Figure 5E). A tumour in the spleen formed by bundles of spindle cells was considered as a high-grade metastasis (Figure 5F).

Dog 353 had two mammary tumours on the right side. In the first gland (R 1) an 8x7x7 cm high-grade combined osteosarcoma was found (Figure 6A), and in gland R 4 a simple schirrhous carcinoma, approximately 2 cm in diameter, adjacent to the nipple was found (not shown). The dog was euthanized 6 months postoperatively due to lung metastases. The lung metastases contained either both cartilage and bone (Figures 6B-F) or cartilage alone (Figures 6G and H). The latter was also valid for metastases in the kidneys (Figure 6I). The x-ray of the skeleton was negative, confirming that the osteosarcoma located in the mammary glands neither originated from the skeleton nor had metastasized there. Further, at autopsy the dog had a simple mammary carcinoma of tubulo-papillary type in both gland L 1 and L 2. In addition, the dog suffered from chronic nephritis, nephrolithiasis and urolithiasis.

The tumours in nude mice formed by the cloned cell line CMT-U353B were, in all but one clone, osteosarcomas with no sign of

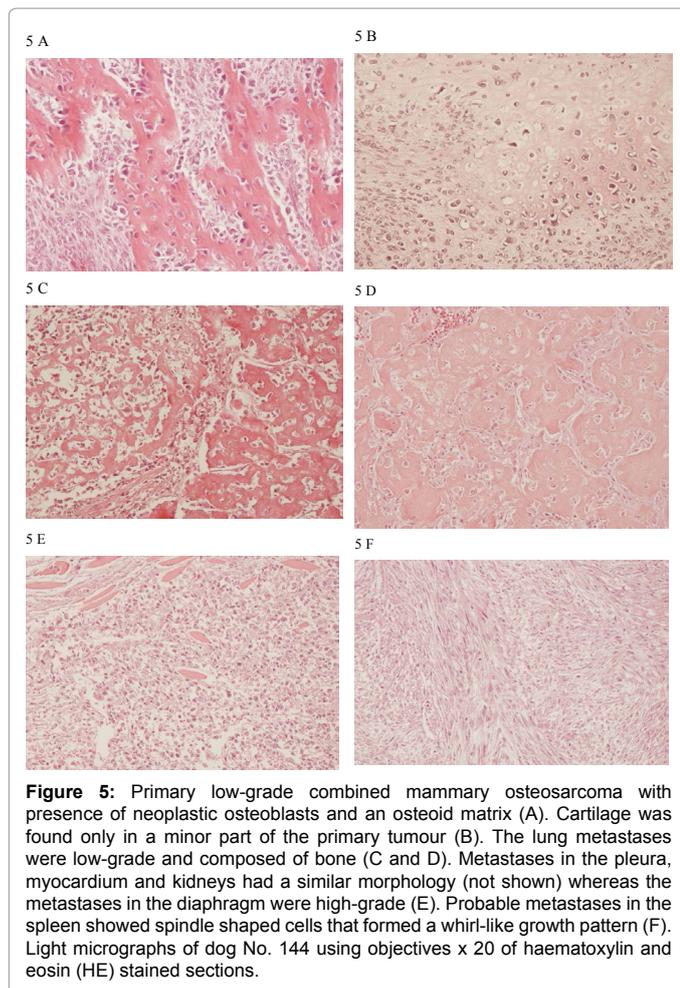


Figure 5: Primary low-grade combined mammary osteosarcoma with presence of neoplastic osteoblasts and an osteoid matrix (A). Cartilage was found only in a minor part of the primary tumour (B). The lung metastases were low-grade and composed of bone (C and D). Metastases in the pleura, myocardium and kidneys had a similar morphology (not shown) whereas the metastases in the diaphragm were high-grade (E). Probable metastases in the spleen showed spindle shaped cells that formed a whirl-like growth pattern (F). Light micrographs of dog No. 144 using objectives x 20 of haematoxylin and eosin (HE) stained sections.

chondroid cells or cartilage. The tumours were low grade (Figures 7A-C) but high-grade tumours also appeared (Figure 7D). The only clone that did not form bone matrix grew as high-grade spindle cell tumours that infiltrated into adjacent peripheral nerves and skeletal muscles (Figures 7E and F).

Discussion

All five of the mammary osteosarcomas studied were of combined type i.e. both neoplastic cartilage and bone tissue were present in the primary tumours. In some tumours, cartilage and bone were adjacently located (Figures 1B, 2F and 6A) whereas in others, bone and cartilage were separated by a distance within the tumour (Figures 4A and B and 5A and B). It appears as if there was a transition from cartilage to bone in some tumours i.e. that the tumour cells had transdifferentiated from chondroblasts to osteoblasts (Figures 2F and 6A). This is in contrast to the results from the experimental studies with inoculated mammary osteosarcoma cells in nude mice (Figures 7A-D), [13] and SCID mice [14] where only bone forming osteosarcomas were observed. The reason for this is unknown and should be explored further. The morphology of the bone forming tumours, with a low-grade, bone matrix rich centre and a high-grade, more cellular rich periphery, was similar in both mice and dogs (Figures 1A and 7A). Metastases in the vessels, both in the primary tumour and in the lung metastases of cases No. O389 and No. 117, contained large amounts of bone matrix in addition to pleomorphic tumour cells (Figure 1C, 1F and 3C). The lung metastases from the

combined mammary osteosarcomas also formed both cartilage and bone (Figure 1D, 1E, 6C and D). Some metastases were surprisingly low-graded, with an abundant presence of bone matrix and few tumour

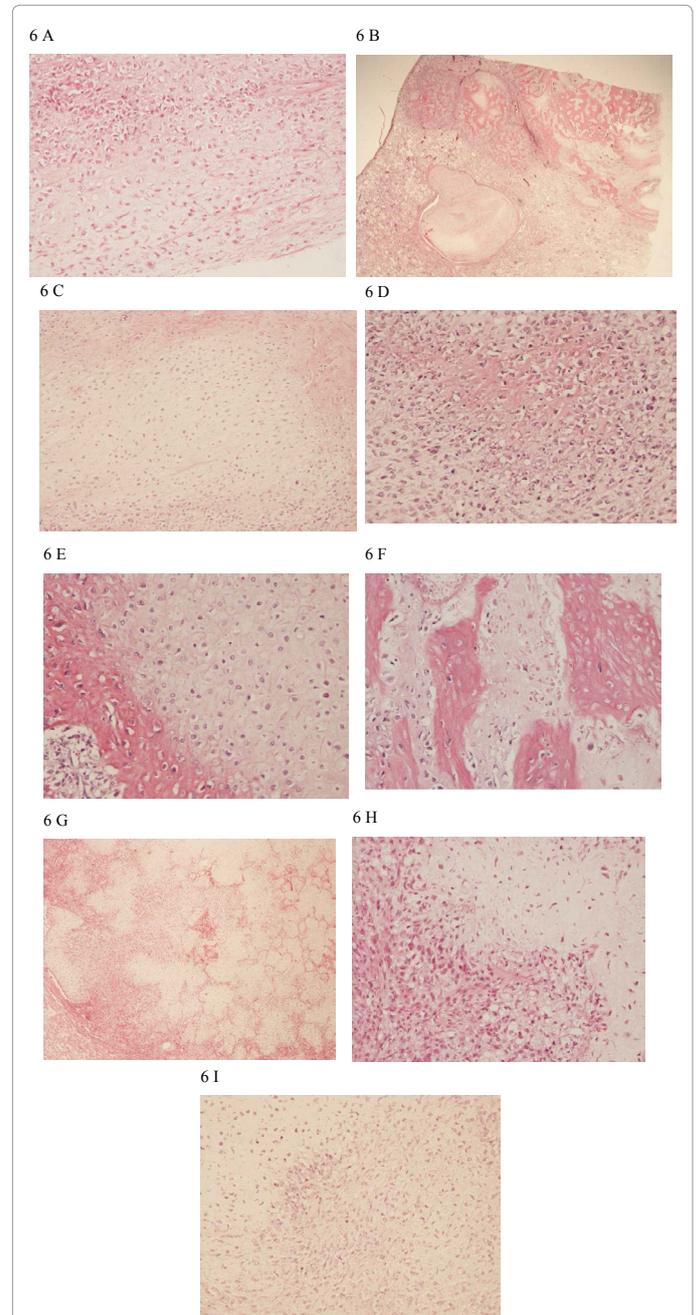


Figure 6: Primary high-grade combined osteosarcoma with neoplastic chondroblasts and osteoblasts with a chondroid and osteoid matrix respectively (A). A lower magnification demonstrating the presence of both cartilage and bone areas in a lung metastasis (B). The letters in Figure B refer to the panels C-F. A well-demarcated area of neoplastic chondrocytes (C) adjacent to tumour cells forming an osteoid matrix (D) and a probable transition between chondrocytes (right side) and osteocytes (left side) were seen, (E). Another lung metastasis showed a low19 grade osteosarcoma (F). In a third lung metastasis (G), less differentiated tumour cells, but still low-grade, forming a chondroid matrix was seen (H). The morphology of this lung metastasis was similar to the kidney metastases (I). Light micrographs of dog No. 353 using objectives x 40 (A); x 1 (B); x 10 (C); x 20 (D-F); x 4 (G) and x 20 (H-I) of haematoxylin and eosin (HE) stained sections.

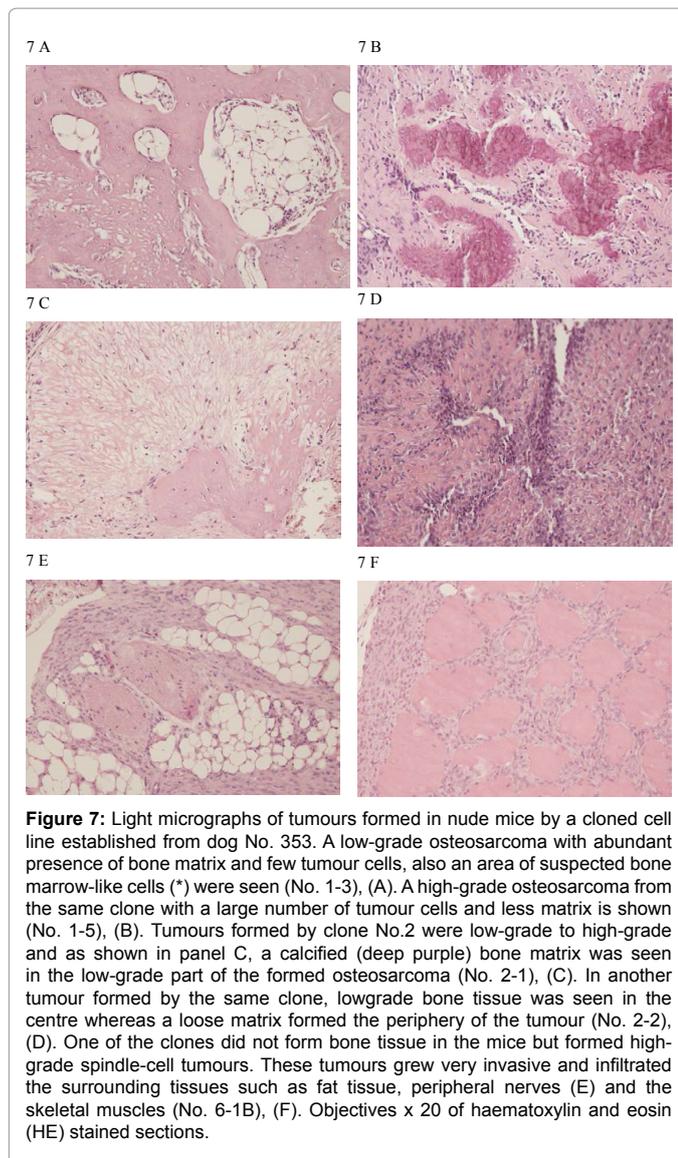


Figure 7: Light micrographs of tumours formed in nude mice by a cloned cell line established from dog No. 353. A low-grade osteosarcoma with abundant presence of bone matrix and few tumour cells, also an area of suspected bone marrow-like cells (*) were seen (No. 1-3), (A). A high-grade osteosarcoma from the same clone with a large number of tumour cells and less matrix is shown (No. 1-5), (B). Tumours formed by clone No.2 were low-grade to high-grade and as shown in panel C, a calcified (deep purple) bone matrix was seen in the low-grade part of the formed osteosarcoma (No. 2-1), (C). In another tumour formed by the same clone, lowgrade bone tissue was seen in the centre whereas a loose matrix formed the periphery of the tumour (No. 2-2), (D). One of the clones did not form bone tissue in the mice but formed high-grade spindle-cell tumours. These tumours grew very invasive and infiltrated the surrounding tissues such as fat tissue, peripheral nerves (E) and the skeletal muscles (No. 6-1B), (F). Objectives x 20 of haematoxylin and eosin (HE) stained sections.

cells (Figures 5C and D). This widely observed but poorly understood phenomenon has been previously reported [6,15].

In general, sarcomas preferentially spread directly to the blood, whereas carcinomas spread via the regional lymph nodes [6,16,17]. In canine mammary osteosarcomas, both metastatic routes have been reported [6,9,18,19]. The reason for lymphogenic spread of canine mammary osteosarcomas is unknown, but it may be linked to the propensity of the tumour cells to invade lymph vessels. Metastasis to the lymph nodes also appears in humans, and is one reason that these tumours are named metaplastic or matrix producing carcinomas. However, it is unknown whether these differences in metastatic spread reflect different origins of these tumours i.e. if they are derived from the mammary parenchyma or stromal tissue [8]. This needs to be investigated further.

Tentative cells that can become neoplastic in the mammary glands are luminal epithelial cells and basally located myoepithelial cells, both derived from the ectodermal germ layer. Hypothetically, some epithelial cells can have a different origin. Further, connective tissue

of mesodermal origin surrounding the ducts and alveoli forming the intralobular and interlobular stromal tissue can also form sarcomas. Interestingly, myoepithelial cells labelled with an anti-CD10 antibody showed the presence of three different CD10 positive cell types in normal canine mammary glands [20]. Thus, there might be more than one cell type located in the basal cell layer as well. Interestingly, it has recently been reported for the first time that tumour initiating cells in human breast sarcoma cells, established from the sarcomatous part of a breast carcinosarcoma, express CD49d^{+/high}, form spheres and give rise to breast sarcomas in NOD/SCID mice [8]. This finding could imply that human breast sarcoma is a true entity and has stem cell-like properties. The need to study human metaplastic breast carcinomas has also been highlighted recently [21].

To compare primary canine carcinomas, fibrosarcomas and osteosarcomas from mammary tumours, we carried out a gene expression study that initially showed that the tumours formed these groups in unsupervised hierarchical clustering [10]. We chose to study malignant monophasic tumours i.e. tumours that are composed of one type of tumour cell, and used Affymetrix Canine Genome 2.0 arrays with 38 000 genes. When we compared the gene expression pattern in the carcinomas compared with the sarcomas by supervised hierarchical clustering, we found a high frequency of embryonic genes in the sarcomas, among them a clear overrepresentation of genes that participate in the formation of the head, such as craniofacial tissues, teeth and nerve tissue. These interesting results clarify some of our previous findings. We then studied primary tumours [22] and cell lines established from different types of canine mammary tumours, and showed that some of the tumours expressed neurofilaments, as demonstrated by immunohistochemistry [23]. In the latter study, cells in primary mammary fibro- and osteosarcomas formed different types of mesenchymal tumours, such as spindle cell tumours, rhabdomyoid, chondroid and leiomyoma-like tumours in nude mice. Our conclusion from that study was that the tumours might originate from pluripotent stem cells. To refine the study we cloned three mammary tumour cell lines; from a carcinoma, a fibrosarcoma/spindle cell tumour and an osteosarcoma. We found a similar plasticity e.g. clones from the spindle cell tumour formed bone tumours in the mice. Further, we also found neurofilament positive cells in the primary spindle-cell tumour and osteosarcoma as well as in one experimental mouse tumour from the osteosarcoma. However, the carcinomas retained their phenotype in the mice although desmoplasia was seen [13]. Taken together, we have seen no evidence of transition between the canine mammary carcinomas and sarcomas. Rather, the sarcomas appear to be very robust with the specific characteristics described above.

Epithelial to Mesenchymal Transition (EMT), which is a normal and reversible process during embryogenesis, is an explanatory model that has been related to a stem cell phenotype in breast cancer [24]. Initiation of the reverse process i.e., mesenchymal to epithelial transition (MET) can be programmed by Klf4. In breast cancer, MET is far less studied than EMT [25]. We have shown that different bone morphogenetic proteins (BMPs) were expressed in the clones from a canine mammary spindle-cell tumour and the canine mammary osteosarcoma, and particularly that BMP-6 was related to bone formation [26]. Interestingly, genes involved in the AKT/PI3K and GLI/Hedgehog signalling pathways have been demonstrated by gene expression arrays of two primary canine mammary osteosarcomas, and by using two benign canine mammary osteomas as controls [27]. In veterinary medicine, there is a general theory that the myoepithelial cells are responsible for the spindle-cell component, at least in complex canine mammary tumours [28]. Due to lack of myoepithelial cell

specific markers, their role is difficult to confirm. However, it is very important to distinguish a primary mammary sarcoma from both a carcinosarcoma and from a metaplastic carcinoma, as the management is different. Thus, finding the cell of origin in mammary sarcomas is critical to understanding mammary gland tumorigenesis.

In previous studies of DNA ploidy, we have shown that the mammary sarcomas are often diploid or near diploid, in contrast to the carcinomas that are most often hypodiploid or hyperdiploid [4,29]. The DNA indices are retained in the metastases [9]. Whether these findings reflect different pathogeneses between canine mammary sarcomas and carcinomas remains to be shown. Another difference between canine mammary osteosarcomas and carcinomas is the fact that only the studied osteosarcomas have a mutated p53 gene [30]. This is also valid for p 53 detected at protein level, which was only seen in the canine mammary osteosarcomas [31,32].

In conclusion, canine mammary combined osteosarcoma metastases were spread haematogenous to the lungs in three dogs and spread via the regional lymph nodes in two dogs. The metastases differed in morphology, both within and between different metastatic sites. Some of the metastases had an even lower grade than the primary tumour. Further, the metastases were of mesenchymal phenotypes although not all of them formed bone tissue. Mammary osteosarcomas are poorly understood tumours, and their pathogenesis and histogenesis are still to be ascertained.

References

- Schneider R, Dorn CR, Taylor DO (1969) Factors influencing canine mammary cancer development and postsurgical survival. *J Natl Cancer Inst* 43: 1249-1261.
- Misdorp W, Else RW, Hellmen E, Limpscomb TP (1999) *Histological Classification of Mammary Tumors of the Dog and the Cat*. Washington D.C. Armed Forces Institute of Pathology in cooperation with the American registry of Pathology and The World Health Organization Collaborating Center for Worldwide Reference on Comparative Oncology. Pp. 5-58.
- Bostock DE (1986) Canine and feline mammary neoplasms. *Br Vet J* 142: 506-515.
- Hellmén E, Bergström R, Holmberg L, Spångberg IB, Hansson K, et al. (1993) Prognostic factors in canine mammary tumors: a multivariate study of 202 consecutive cases. *Vet Pathol* 30: 20-27.
- Langenbach A, Anderson MA, Dambach DM, Sorenmo KU, Shofer FD (1998) Extraskeletal osteosarcomas in dogs: a retrospective study of 169 cases (1986-1996). *J Am Anim Hosp Assoc* 34: 113-120.
- Misdorp W, Cotchin E, Hampe JF, Jabara AG, von Sandersleben J (1971) Canine malignant mammary tumours. I. Sarcomas. *Vet Pathol* 8: 99-117.
- Kusafuka K, Muramatsu K, Kasami M, Kuriki K, Hirobe K, et al. (2008) Cartilaginous features in matrix-producing carcinoma of the breast: four cases report with histochemical and immunohistochemical analysis of matrix molecules. *Mod Pathol* 21: 1282-1292.
- Rakha EA, Tan PH, Shaaban A, Tse GM, Esteller FC, et al. (2013) Do primary mammary osteosarcoma and chondrosarcoma exist? A review of a large multi-institutional series of malignant matrix-producing breast tumours. *Breast* 22: 13-18.
- Hellmén E, Svensson S (1995) Progression of canine mammary tumours as reflected by DNA ploidy in primary tumours and their metastases. *J Comp Pathol* 113: 327-342.
- Wensman H, Göransson H, Leuchowius KJ, Strömberg S, Pontén F, et al. (2009) Extensive expression of craniofacial related homeobox genes in canine mammary sarcomas. *Breast Cancer Res Treat* 118: 333-343.
- Goldschmidt M, Peña L, Rasotto R, Zappulli V (2011) Classification and grading of canine mammary tumors. *Vet Pathol* 48: 117-131.
- Dolka I, Sapieryż A, Ski R, KrÅ M (2013) Retrospective study and immunohistochemical analysis of canine mammary sarcomas. *BMC Vet Res* 9: 248.
- Wensman H, Flama V, Pejler G, Hellmén E (2008) Plasticity of cloned canine mammary spindle cell tumor, osteosarcoma and carcinoma cells. *Vet Pathol* 45: 803-815.
- Kawabata A, Yamamoto K, Lan NT, Uchida K, Yamaguchi R, et al. (2006) Establishment and characterization of a cell line, MCO-Y4, derived from canine mammary gland osteosarcoma. *J Vet Med Sci* 68: 1047-1053.
- Misdorp W, Hart AA (1979) Canine mammary cancer. II. Therapy and causes of death. *J Small Anim Pract* 20: 395-404.
- Misdorp W, Cotchin E, Hampe JF, Jabara AG, von Sandersleben J (1972) Canine Malignant Mammary Tumours II. Adenocarcinomas, Solid Carcinomas and Spindle Cell Carcinomas. *Veterinary Pathology Online* 9: 447-470.
- Misdorp W, Cotchin E, Hampe JF, Jabara AG, Von Sandersleben J (1973) Canine malignant mammary tumors. 3. Special types of carcinomas, malignant mixed tumors. *Vet Pathol* 10: 241-256.
- Misdorp W, Hart AA (1976) Prognostic factors in canine mammary cancer. *J Natl Cancer Inst* 56: 779-786.
- Misdorp W, Hart AA (1979) Some prognostic and epidemiologic factors in canine osteosarcoma. *J Natl Cancer Inst* 62: 537-545.
- Sánchez-Céspedes R, Suárez-Bonnet A, Millán Y, Guil-Luna S, Reymundo C, et al. (2013) Use of CD10 as a marker of canine mammary myoepithelial cells. *Vet J* 195: 192-199.
- Weigelt B, Eberle C, Cowell CF, Charlotte KY, Reis-Filho JS (2014) Metaplastic breast carcinoma: more than a special type, 14: 147-148.
- Hellmén E, Lindgren A (1989) The expression of intermediate filaments in canine mammary glands and their tumors. *Vet Pathol* 26: 420-428.
- Hellmén E, Moller M, Blankenstein MA, Andersson L, Westermark B (2000) Expression of different phenotypes in cell lines from canine mammary spindle-cell tumours and osteosarcomas indicating a pluripotent mammary stem cell origin. *Breast Cancer Res Treat* 61: 197-210.
- Scheel C, Weinberg RA (2012) Cancer stem cells and epithelial-mesenchymal transition: concepts and molecular links. *Semin Cancer Biol* 22: 396-403.
- Chen J, Liu J, Yang J, Chen Y, Chen J, et al. (2011) BMPs functionally replace Klf4 and support efficient reprogramming of mouse fibroblasts by Oct4 alone. *Cell Res* 21: 205-212.
- Wensman H, Heldin NE, Pejler G, Hellmén E (2009) Diverse bone morphogenetic protein expression profiles and smad pathway activation in different phenotypes of experimental canine mammary tumors. *PLoS One* 4: e7133.
- PawÅowski KM, Majewska A, Szyszko K, Dolka I, Motyl T, et al. (2011) Gene expression pattern in canine mammary osteosarcoma. *Pol J Vet Sci* 14: 11-20.
- Peña L, Gama A, Goldschmidt MH, Abadie J, Benazzi C, et al. (2014) Canine mammary tumors: a review and consensus of standard guidelines on epithelial and myoepithelial phenotype markers, HER2, and hormone receptor assessment using immunohistochemistry. *Vet Pathol* 51: 127-145.
- Hellmén E, Lindgren A, Linell F, Matsson P, Nilsson A (1988) Comparison of histology and clinical variables to DNA ploidy in canine mammary tumors. *Vet Pathol* 25: 219-226.
- Van Leeuwen IS, Hellmén E, Cornelisse CJ, Van den Burgh B, Rutteman GR (1996) P53 mutations in mammary tumor cell lines and corresponding tumor tissues in the dog. *Anticancer Res* 16: 3737-3744.
- Inoue M, Shiramizu K (1999) Immunohistochemical detection of p53 and c-myc proteins in canine mammary tumours. *J Comp Pathol* 120: 169-175.
- Haga S, Nakayama M, Tatsumi K, Maeda M, Imai S, et al. (2001) Overexpression of the p53 gene product in canine mammary tumors. *Oncol Rep* 8: 1215-1219.