

CANINE EXTRASKELETAL OSTEOSARCOMA OF THE SALIVARY GLAND

Theerayuth Kaewamatawong* Anudep Rungsipipat* and Suwicha Chutatep**

Abstract

Theerayuth Kaewamatawong* Anudep Rungsipipat* and Suwicha Chutatep**

CANINE EXTRASKELETAL OSTEOSARCOMA OF THE SALIVARY GLAND

An 8 year-old male mongrel dog and 12 year-old male German Shepherd had a 10 x 10 cm mass and 15 x 10 cm mass located in the mandibular region. The encapsulated firm white masses had multiple cystic-like nodules filled with mucous. Histopathology revealed clusters of pleomorphic, large, round to oval shaped cells, irregular ovoid to spindle nuclei with highly mitotic figures that showed osteoid and bony formation. The neoplastic cells were immunoreactive in some parts for vimentin and cytokeratin but not for desmin, α -SMA and S-100 protein. Hyperplastic salivary gland cells were also present. Electron microscopy revealed prominent nucleoli with an abundant, dilated, rough, endoplasmic reticulum. A final diagnosis of extraskeletal osteosarcoma originating from interstitial cells of the salivary gland was made.

Key words : dog, extraskeletal osteosarcoma, salivary gland

*** Department of Veterinary Pathology,** Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University, Bangkok, Thailand. 10330**

* ภาควิชาพยาธิวิทยา **ภาควิชาศัลยศาสตร์ คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330

บทคัดย่อ

ธีระยุทธ แก้วอมตวงศ์* อนุเทพ รังสีพิพัฒน์* สุวิชา จุฑาทะพา**

เนื้องอกของต่อมน้ำลายชนิด extraskelatal osteosarcoma ในสุนัข

สุนัขพันธุ์ผสม เพศผู้ อายุ 8 ปี และพันธุ์เยอรมันเชพเพิร์ด เพศผู้ อายุ 12 ปี พบก้อนเนื้องอกที่ บริเวณต่อมน้ำลายบริเวณขากรรไกรล่าง ขนาด 10 x 10 ซม. และ 15 x 10 ซม. ตามลำดับ มีลักษณะเป็นก้อนเนื้อแน่นสีขาว มีถุงหุ้มภายนอก และภายในมีการสร้างลักษณะคล้ายถุงน้ำบรรจุเมือกปนเลือด ลักษณะทางจุลพยาธิวิทยาพบเซลล์เนื้องอกรูปร่างกลม หรือรีขนาดใหญ่ นิวเคลียสกลมหรือรูปกระสวยไม่แน่นอน มีการสร้างเนื้อเยื่อคล้ายกระดูกและกระดูก มีดัชนีการแบ่งตัวสูง ผลอิมมูโนโนฮิสโตเคมีพบว่าเซลล์เนื้องอกให้ผลบวกต่อ vimentin และ cytokeratin และให้ผลลบต่อ desmin, α -SMA และ S-100 protein ผลการศึกษาทางจุลทรรศน์อิเล็กตรอนพบนิวเคลียสของเซลล์เนื้องอกมีนิวคลีโอไลต์เด่นชัดและภายในเซลล์มีการขยายตัวของเอนโดพลาสมิกเรติคูลัมชนิดหยาบเป็นจำนวนมาก การวินิจฉัยขั้นสุดท้ายคือ extraskelatal osteosarcoma ในสุนัขซึ่งคาดว่าต้นกำเนิดมาจากเซลล์ interstitial ของต่อมน้ำลาย

คำสำคัญ : สุนัข extraskelatal osteosarcoma ต่อมน้ำลาย

Introduction

Neoplasms of the salivary gland are rare in dogs and cats forming only 0.09% of all evaluated specimens (Carberry et al., 1988). Salivary gland neoplasms have numerous types and come under various classification schemes. Spangler and Culbertson (1991) reviewed the classification of salivary gland tumors as malignant adenocarcinomas, malignant mixed salivary tumors, accessory papillary adenomas, cystic adenomas, fibrosarcomas and lymphosarcomas. Extraskelatal osteosarcoma is a mesenchymal neoplasm and rarely occurs in dogs and other domestic animals. The tumor has been reported as a primary tumor in visceral organs such as the spleen, mammary gland, skin, lung, intestine, liver and the soft tissue of the extremities (Jabara and McLeod, 1989; Schena et al., 1989; Kuntz et al., 1998; Langenbach et al., 1998). Patnaik's (1990) review showed that in about

86% of such cases in dogs, the tumor developed in the visceral organs and in 14% developed in the soft tissue of the extremities. Previous reports of extraskelatal osteosarcoma of mandibular salivary gland in dog are rare (Thomsen and Myers, 1999). In this paper, we describe the gross pathology, the radiographic, histologic, immunohistochemical and electron microscopic information of extraskelatal osteosarcoma of mandibular salivary gland, in two dogs.

Materials and Methods

Case histories: Two dogs were submitted to the small animal hospital, Faculty of Veterinary Science, Chulalongkorn University, suffering from hypersecretion of mucous, oronasal discharge and difficulty in chewing and swallowing. The first case was a 8-year-old mongrel male dog presenting a firm subcutaneous, swelling mass, 2.5 x 4 cm in size in the right mandibular region. A radiographic image

showed a soft tissue swelling in the right mandibular area and focal white opacity at the ventral edge without any evidence of bone involvement (Figure 1). Some parts of the mass were surgically removed and histopathology showed a salivary mucocele. One month later, after the surgery, the mass beside the salivary mucocele had grown to 10 x 10 cm compressing trachea and causing tracheal and oesophageal collapse. The owner requested euthanasia. The second case was a 12-year-old, male, German Shepherd dog presenting a 15 x 10 cm, firm mass in the left mandibular area and showing signs of depression, anorexia and hypersalivation. Radiography showed a soft tissue swelling in the left mandibular region with no evidence of bone involvement, similar to the previous case. A biopsy was performed.

Histopathology: The selected tissues were fixed in 10% neutral buffered formalin, processed for histology, embedded in paraffin, sectioned 4 µm thick and stained with Hematoxylin and Eosin (HE). Other sections were stained with Masson's trichrome (MT), Periodic acid Schiff (PAS) and Toluidine blue (TB).

Immunohistochemistry: Representative 4 µm paraffin sections were stained by the standard avidin biotin complex immunoperoxidase method (ABC kit®, Dako, Denmark) with the following primary antibodies; mouse monoclonal anti-vimentin, rabbit polyclonal anti-cytokeratin, rabbit polyclonal anti-S 100 protein, mouse monoclonal anti-alpha smooth muscle actin (α-SMA) and mouse monoclonal anti-desmin (Dako, Denmark). The sections were counterstained with Meyer's hematoxylin, visualized by chromogen (DAB; 3,3' diaminobenzidine tetrahydrochloride) and examined under a light microscope.

Electron microscopy: Portion of tissues were fixed in 1 % glutaraldehyde and postfixed in 1% osmium tetroxide, dehydrated in graded alcohol and propylene oxide, and embedded in epoxy plastic. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a TEM (JEM 200 CX, Hitachi, Japan) according to conventional methods.

Results

Cytologic images revealed small, round, hyperplastic resembling epithelial cells lining the salivary gland and small clusters of spindle shape cells with large round to oval nuclei and 2-3 prominent nucleoli (Figure 2). The cytoplasm was stained deeply basophilic and contained multiple small vacuoles.

Macroscopic findings

The 8 year old mongrel had a firm white encapsulated mass, 10 cm in diameter, located in the right mandibular area. The cut surface showed multiple cystic formation containing mucous exudate and blood clots. The major lymph nodes of the head and neck regions appeared normal. Other lesions noted were fatty degeneration of liver, tape worms and heartworm infestation together with senile valvular endocardiosis.

The German Shepherd dog had an encapsulated mass measuring about 15x10 cm that extended from the base of the left aural pinna to the mandibular angle. The mass was white and firm in consistency. The cut surface consisted of an invasive mass, infiltrating the adjacent tissue in that area. The regional lymph nodes appeared normal. Other lesions present were pulmonary oedema with mild congestion in all the lobes and prostate gland enlargement.

Microscopic findings

Histopathology of the first case revealed a densely compact arrangement of large polyhedral shape cells which had pleomorphic, large, round to oval nuclei with 1-2 prominent nucleoli. The mitotic figures contained 2-3 cells/HPF (Figure 3). The cytoplasm was pale and homogeneous eosinophilic staining. Some areas of the mass osteoblastic cells accompanied by giant tumor cells (Figure 4). A necrotic area could be seen at the edge of mass and the invasive nodules. In the second case, the main characteristic of the tumor cells was similar to the first case but had an increase of mainly cuboidal acini in an epithelial arrangement that could be recognized as hyperplastic salivary glands (Figure 5). These were separated from the neoplastic cells by a thick fibrous tissue stroma. The PAS stain also showed positive for mucous glands, the MT stain showed a highlighted osteoid matrix with a fibrous tissue stroma (Figure 6).

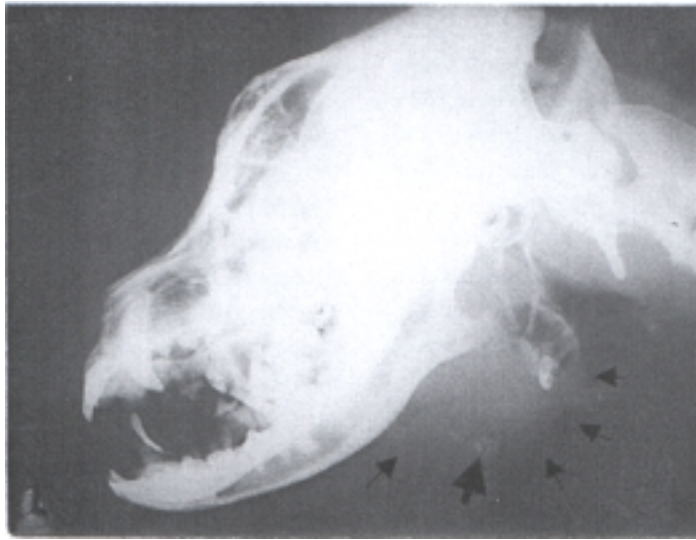


Figure 1. The Radiographic image showed a soft tissue swelling in the right mandibular region (small arrow) with focal opacity at it's center (large arrow) and no evidence of bone involvement.

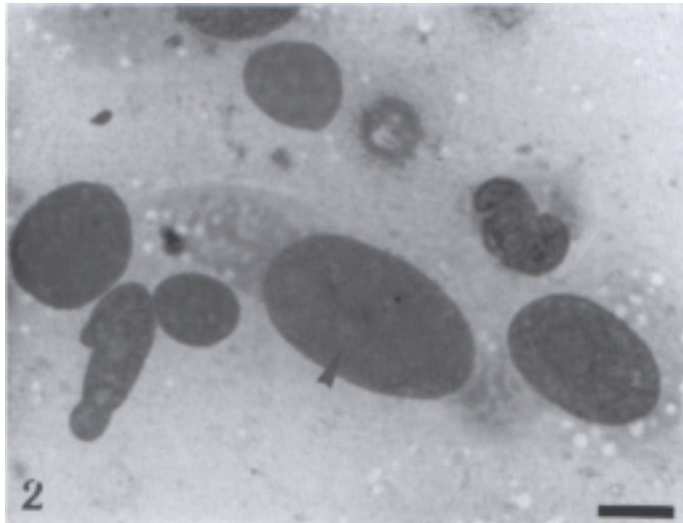


Figure 2. Cytology of the second case revealed small clusters of spindle shaped cells that had oval nuclei with 2 –3 prominent nucleoli (arrowhead). The cytoplasm was strongly basophilic staining with small vacuoles (Giemsa, bar = 17 μ m).

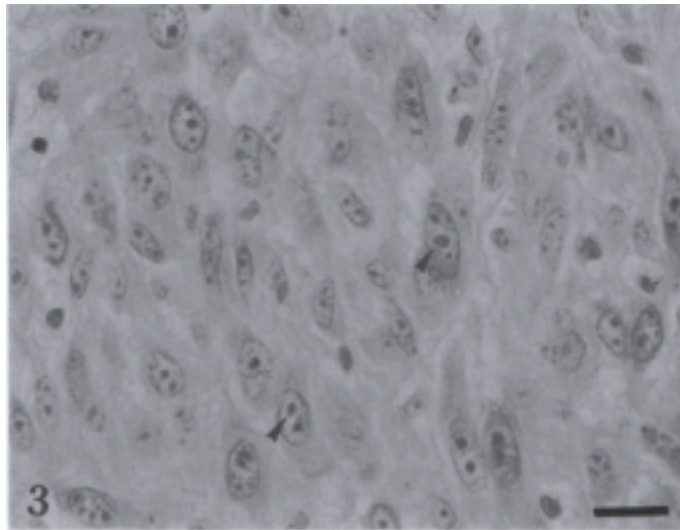


Figure 3. Histopathology on the first case showed large polygonal shaped cells, pleomorphic large oval nuclei with 1-2 prominent nucleoli (arrow head , H&E, bar = 25 μ m).

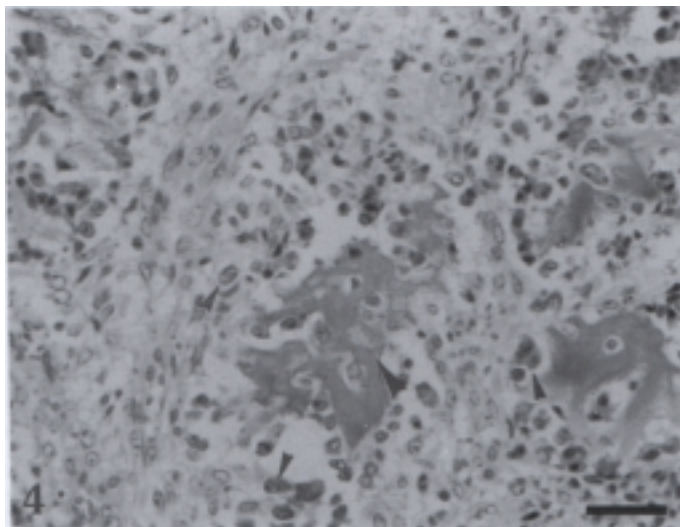


Figure 4. The osteoid matrix (large arrowhead) contained an irregular arrangement of neoplastic cells and giant tumor cells (small arrowhead) located around the matrix (HE, bar = 50 μ m).

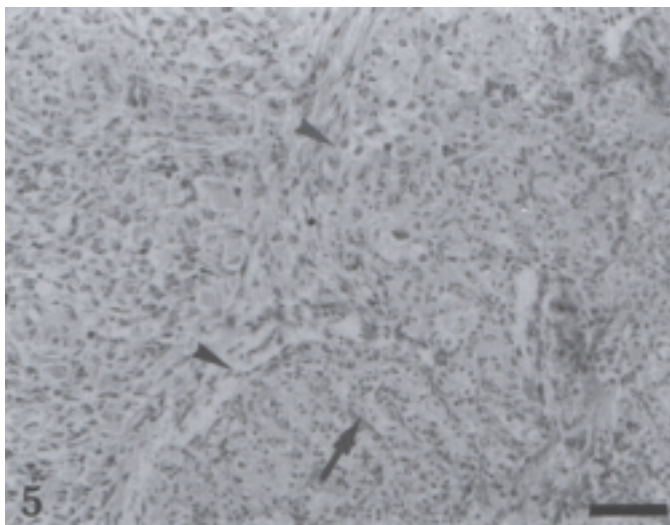


Figure 5. Salivary gland hyperplasia (arrow) was separated from the neoplastic cells by fibrous connective tissue stroma (arrowhead, HE, bar = 100 μ m).

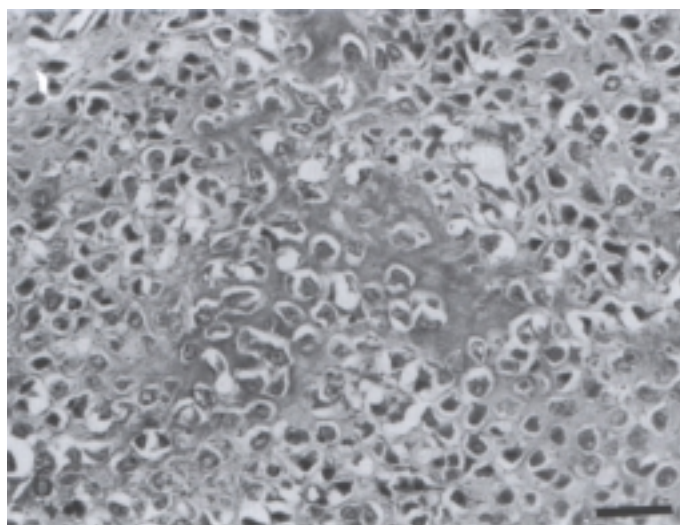


Figure 6. In the first case, positive staining for osteoid matrix is shown as blue (MT, bar = 50 μ m).

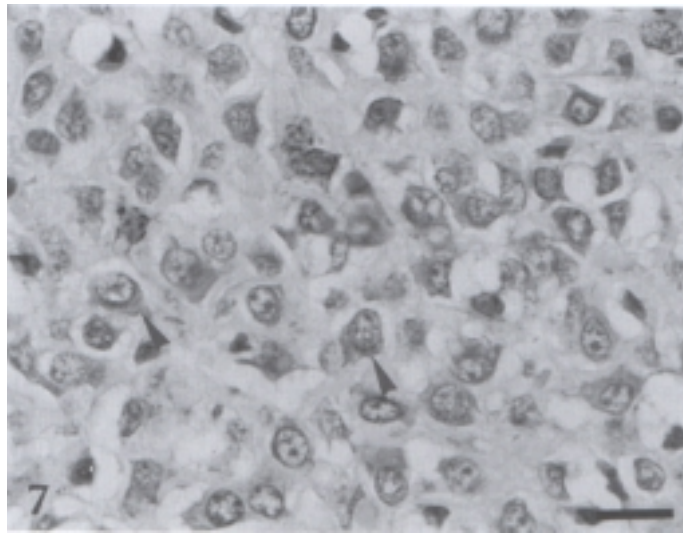


Figure 7. In both cases, the neoplastic cells cytoplasm was immunoreactive with vimentin (arrowhead, ABC, counterstain with Meyer's hematoxylin, bar = 25 μ m).

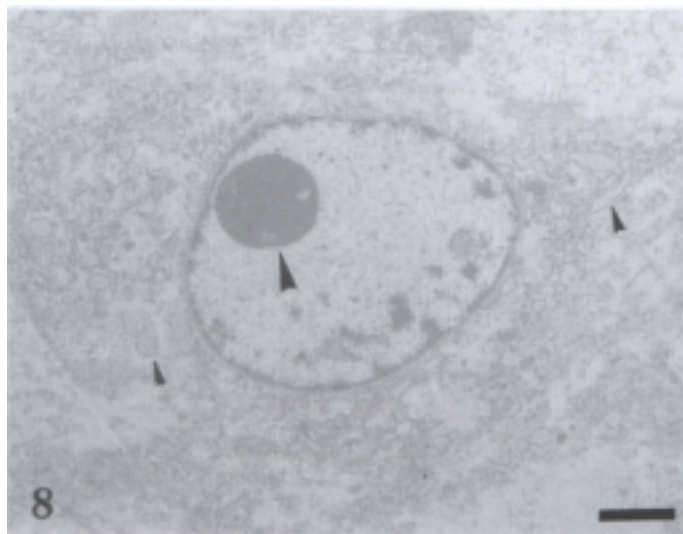


Figure 8. The ultrastructure of the tumor cell revealed prominent nucleoli with dispersion of the nuclear material (large arrowhead) and dilated rough endoplasmic reticulum (small arrowhead, bar = 1 μ m).

and the TB stain showed cytoplasmic granules in the osteoid cells.

Upon necropsy, tissue samples were selected from many areas of the tumor and various visceral organs. The morphologic characteristics of the mass were similar to those seen in the previous sections but the distribution of the neoplastic part of the mass was more scattered than in the biopsy. Other sections from various visceral organs showed no metastatic lesions.

Immunohistochemistry: In both cases, immunohistochemical images showed a positive reaction to vimentin by the cytoplasm of the osteoblastic tumor cells (Figure 7) that was distributed throughout the mass. In the second case, the cytoplasm of the cuboidal epitheliums of the salivary gland was positive when stained with cytokeratin. For the other antibodies, immunohistochemistry was negative.

Electron microscopy

The characteristic electron microscope features of the neoplastic cells were prominent nucleoli with a dispersion of nuclear materials and abundant, dilated, rough, endoplasmic reticulum in the cytoplasm (Figure 8).

Discussion

Extraskelatal osteosarcomas are mesenchymal soft tissue neoplasms found in various visceral organs but rare in domestic animals and human beings. In these two cases, the main characteristics of the tumor masses were a gray-white color and lobulated the ossified mass and had necrotic areas and a cyst like structure that was similar to that in other previous reports (Patnaik, 1990). The histological characteristics of this tumor showed small to large, oval, spindle to polygonal shaped cells with large vesiculated nuclei, one or more large nucleoli and eosinophilic or clear cytoplasm. Some of the osteoids were mineralized in the same way as the typical tumor cells described in this report and which were recognized as osteoblastic cells. Some multinucleated tumor cells are occasionally seen in a poorly differentiated osteosarcoma and this may be helpful for diagnosis but the small number of giant tumor cells which were evident in these

cases how them to be the well-differentiated type (Patnaik, 1990; Thomsen and Myers, 1999).

Extraskelatal osteosarcomas may be metastases from primary skeletal tumors, fibrosarcomas with osseous metaplasia, osteochondromas, chondrosarcomas and other mesenchymal tumors. In these cases, there was no evidence of a primary skeletal tumor which ruled out the metastases (Moulton, 1990). The cytological characteristics of osseous metaplasia from a fibrosarcoma are uniform osteoblasts with mature osteoids which contrasts with the irregular like osteosarcoma and the fascicular, herring-bone pattern that predominated in fibrosarcomas (Fechner and Mills, 1992). The absence of chondrocytes and cartilagenous components in these cases eliminates osteochondromas and chondrosarcomas (Misdorp and Van Der Heal, 1976). Differentiation between extraskelatal osteosarcomas and other mesenchymal tumors may be confirmed by the presence of abundant osteoid in extraskelatal osteosarcoma.

Extraskelatal osteosarcomas in the dog are malignant tumors and usually metastasises to various visceral organs depending on the stage of tumor. In comparison to these cases, these tumors seem to be a well differentiated type and no metastatic lesions could be observed.

In previous reports of extraskelatal osteosarcoma, immunohistochemical results showed them to be immunoreactive to vimentin and actin but not to keratin, desmin and S-100, suggesting they originate from myogenic cells. In these cases, the tumor cells were immunoreactive to vimentin which supported a mesenchymal origin and to cytokeratin that stained the areas where there were cuboidal hyperplastic epithelial cells of the salivary gland. This situation supported the theory origin of the tumor was mainly from the mesenchyme. A pluripotential cell can differentiate along it's developmental pathway to become a cell which exhibits functions and morphology unique to it's particular lineage. This theory elucidates how a bone tumor such as an osteosarcoma can occur in an organ or tissue that does not contain bony tissue (Thomsen and Myers, 1999). The ultrastructure, the characteristics of the neoplastic cells showing prominent nucleoli and dispersion of nuclear material that

indicated a high level of activity by the cell. They had abundant, dilated, rough, endoplasmic reticulum which resembled previous reports of the cell line from canine osteosarcomas (Kadosawa et al., 1994).

By the morphologic features, the histopathologic examinations, immunohistochemical staining characteristics, and electron microscopy of these two cases they fitted the criteria of extraskelatal osteosarcomas in the salivary gland. The prognosis was always poor and depended on the aggressive local behavior, bone involvement and visceral organ compression. Some previous reports suggested surgery for such neoplastic tissues but there was always the risk of metastasis that might have adversely affected the survival times of the patients (Thomsen and Myers, 1999).

Acknowledgements

This work was supported by a development grants for new faculty/researchers, 2000 Chulalongkorn University. We thank Dr. Pasakorn Briksawan, Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University for the radiography.

References

- Carberry, C.A., Flanders, J.A., Harvey, H.J. and Ryan, A.M. 1988. Salivary gland tumors in dogs and cats: a literature and case review. *J. Am. Anim. Hosp. Assoc.* 24: 561-567.
- Fechner, R.E. and Mills, S.E. 1992. Tumors of the Bones and Joints; Atlas of Tumor Pathology. Armed Forces Institute of Pathology, Washington D.C. p. 145-163.
- Jabara, A.G. and McLeod, J.B. 1989. A primary extraskelatal osteogenic sarcoma arising of a new cell line from a canine osteosarcoma. *J. Vet. Med. Sci.* 56(6): 1167-1169.
- Kadosawa, T., Nozaki, K., Sasaki, N. and Takeuchi, A. 1994. Establishment and characterization of new cell line from a canine osteosarcoma. *J. Vet. Med. Sci.* 56(6): 1167-1169.
- Kuntz, C.A., Dernell, W.S., Powers, B.E. and Withrow, S. 1998. Extraskelatal osteosarcomas in dogs: 14 cases. *J. Am. Anim. Hosp. Assoc.* 34(1): 26-30.
- Langenbach, A., Anderson, M.A., Dambach, D.M., Sorenmo, K.U. and Shofer, F.D. 1998. Extraskelatal osteosarcomas in dogs : a retrospective study of 169 cases (1986-1996). *J. Am. Anim. Hosp. Assoc.* 34(2):
- Misdorp, W. and Van Der Heal, R.O. 1976. Tumors of bones and joints. *Bulletin of the world health organization.* 53(2): 265-282.
- Moulton, J.E. 1990. Tumors of bone and cartilage. *Tumors in Domestic Animals.* University of California Press, Berkley, CA. p 157-227.
- Patnaik, A.K. 1990. Canine extraskelatal osteosarcoma and chondrosarcoma: a clinicopathologic study of 14 cases. *Vet. Pathol.* 27:46-55.
- Schena, C.J., Stickle, R.L., Dunstan, R.W., Trapp, A.L., Reimann, K.A., White, J.V., Killingsworth, C.R. and Hauptman, J.G. 1989. Extraskelatal osteosarcoma in two dogs. *J. Am. Vet. Med. Assoc.* 194(10): 1452-1456.
- Spangler, W.L. and Culbertson, M.R. 1991. Salivary gland disease in dogs and cats: 245 cases (1985-1988). *J. Am. Vet. Med. Assoc.* 198(3): 465-469.
- Thomsen, B.V. and Myers, R.K. 1999. Extraskelatal osteosarcoma of the mandibular salivary gland in a dog. *Vet. Pathol.* 36: 71-73.