# *ABSTRACT*

Spontaneous mammary tumors are the most common neoplasia in the female dog and have a high biological and histomorphological heterogeneity. Approximately one-half of all mammary tumors in dogs are malignant and have a great potential to metastasize to the regional lymph nodes or other organs such as the lungs.

Malignant transformation is associated with abnormal glycosylation, resulting in the synthesis and expression of altered carbohydrates determinants.

In humans, the majority of breast cancer research is conducted using established breast cancer cell lines as *in vitro* models because they have the ability to proliferate indefinitely. However, the interaction between the tumor and the host organism must be taken into consideration, so the results need to be confirmed using animal models.

In order to have an in depth view of the biology of canine mammary tumors, two experimental models, one *in vitro* and another *in vivo*, were established.

In the first part of this work, three canine mammary carcinoma cell lines were established - one complex adenoma and two complex carcinomas - using fragments of tumors excised during a surgical procedure performed on a female dog. After the *in vitro* immunostaining for intermediate filaments, the presence of epithelial and mioepithelial cells was determined. When these cell lines were heterotlansplanted in *nude* mice it was noted that they were not tumorigenic.

In the second part of the work an *in vitro* and *in vivo* characterization of a previously established cell line was made, derived from a canine mammary ductular carcinoma. These cells adhered to the bottom of the flask as a compact thin monolayer, had a doubling time of 32,6±1,4 hours and when they were grown in agar they formed a unique large aggregate. Cell motility assay showed that these cells conserved an epithelial-like architecture and had the ability to move between the edges of an artificial wound in a compact front of migration, with the cells moving unidirectionally. These cells had immunoreactivity for E-cadherin and showed a high proliferative index. A panel of antibodies for intermediate filaments was used to evaluate the origin of the cell line and it showed intense immunoreactivity for AE1/AE3 keratin which is a characteristic feature of epithelial cells. CK14 was expressed in 25-50% of the cells and vimentin also showed a high level of expression, which can be normal in culture tissues. These cells also immunostained for the carbohydrates $sLe^x$, $Le^x$ and $Le^a$, and this

aberrant expression may also play a fundamental role in the molecular mechanisms of metastization to distant organs, facilitate positive interactions within the target organ and could be useful as a prognostic tumour marker.

*In vivo* assays showed that these cells grew when inoculated subcutaneously in the mammary fat pad of female *nude* mice. Tumour masses were histologically identical to the mammary tumour lesions they derived from, and when heterotransplanted tumours were cultured, the expression of intermediate filaments and carbohydrates was not altered. To look for metastatic target tissues we performed an intravenous injection in the tail vein of the mice. These cells metastasized to lymph nodes, lungs, heart, spleen, kidney and liver. The tumorigenicity and metastization observed in *nude* mice, as well as the motility of this cell line *in vitro* are suggestive of an aggressive phenotype for these cells.

On the whole, the results obtained from this work suggest that canine mammary tumours are good models for the study of human breast cancer.