Serum Galectin-3 Levels in Dogs with Metastatic and Non-metastatic Mammary Tumors

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Abstract. Galectin-3 is implicated in tumor progression and metastasis. High levels of galectin-3 have been reported in intravasated cells in primary and metastatic tumor sites of canine malignant mammary tumors (CMMT). Nevertheless, it is still unknown whether this increase is limited to the site of the lesion or if it is a systemic feature. To better understand the pattern of the expression of galectin-3 and to investigate the possibility of using serum galectin-3 levels as a relevant biomarker in this disease, galectin-3 concentrations were determined in a series of sera from CMMT-bearing female dogs. None of the dogs included in the study had detectable metastases at the time of presentation. Animals were retrospectively divided into two groups dependent on whether or not they developed metastatic lesions during a 25-month follow-up period. Samples were collected from all dogs before surgery, 1 month after resection of the primary tumor and every 3 months during the postoperative period. Galectin-3 levels were significantly higher 1 month after than at the time of surgery (p=0.0058). Higher galectin-3 was found in samples collected 7 (p=0.0007), 10 (p=0.0061) and 13 months (p=0.0052) after surgery from dogs of the metastatic group when compared to those remaining free of development of detectable metastases. In conclusion, increased serum galectin-3 levels seem to be present in both metastatic and non-metastatic cases during the postoperative period, however, while in non-metastatic cases the values tend to return to baseline levels after surgery, in metastatic cases, levels remain persistently elevated.

Galectin-3 is a member of the galectin family of carbohydrate binding proteins, and is implicated in multiple functions such as cell–cell and cell–extracellular matrix (ECM) adhesion, angiogenesis promotion, cell proliferation and apoptosis resistance, the latter actions ultimately facilitating tumour progression and metastasis (1). In canine malignant mammary tumors (CMMT), galectin-3 and its binding sites are up-regulated in vessel-invading tumor cells, indicating a possible role in the metastatic process (2). Understanding the regulation and the dynamics of galectin-3 and its partners related to the process of metastasis might be of value in the design of new strategies for diagnosis and treatment. Prognostic value of serum galectin-3 levels has been described in human melanoma, bladder and lung cancer and in breast cancer (3-5).

We investigated the possibility of using serum galectin-3 levels as a cancer biomarker in CMMT.

Materials and Methods

Galectin-3 concentration was measured in serum samples of female dogs before and after tumor resection. Serum samples from 21 female dogs bearing CMMT, with an average age of 10.58 years, were analyzed. None of the dogs included in the study had detectable metastases at the time of presentation. Animals were retrospectively divided into two groups dependent on whether or not they developed metastatic lesions at 25-month follow-up period.

Serum samples were collected before (T0) and 1 month after primary tumor resection (T1), as well as every 3 months for the subsequent months or until the time of death (T3 to T25). Enzyme-linked immunosorbent assay (ELISA) was performed to measure serum galectin-3 levels. Briefly, to determine serum galectin-3 concentration 96 well-plates were pre-coated with a non-biotinylated anti-galectin-3 monoclonal antibody, M3/38 (eBioScience, San Diego, CA, USA) (0.25 μl/well), overnight. Next, standards (prepared with increasing dilutions of human recombinant galectin-3) (Sigma-
Aldrich, St. Louis, MO, USA) and samples were added to the wells and incubated. Upon washing, incubation with biotinylated M3/38 and streptavidin-horse radish peroxidase followed. All incubations were performed at 37°C. After vigorous washing, 3,3',5,5'-tetramethylbenzidine was added to the wells. The reaction was subsequently stopped with a sulphuric acid-based stop reagent. Absorbance was measured at 562 nm.

GraphPad Prism 5 package software (GraphPad Software, Inc. La Jolla, CA 92037 USA) was used for statistical analysis. Despite the total follow-up period having been 24 months, animal death precluded statistical comparison between groups beyond 13 months.

Results

Serum galectin-3 levels in the 21 dogs before the surgery rayed between 0 and 6.32 ng/ml (median=1.13 ng/l), after surgery between 0 and 9.93 ng/ml (median=2.22 ng/ml) and 3 months later between 0 and 9.11 ng/ml (median, 1.48 ng/ml). Serum galectin-3 levels 1 month after surgery were significantly higher than before surgery ($p=0.0058$) (Figures 1 and 2). Overall, galectin-3 levels 4 months after surgery appeared to be lower than at 1 month, but this was not statistically significant (Table I).

Serum galectin-3 of dogs that developed metastases was higher when compared to that of those that did not. This difference was statistically significant at 7 ($p=0.0007$), 10 ($p=0.0061$) and 13 months ($p=0.0052$) of the postoperative period (Table II). Galectin-3 levels increased before the visible presence of metastases in two out of six dogs with metastases.

Discussion

Our work showed that serum galectin-3 levels in all dogs with CMMT were significantly higher at 1 month after surgery. This may be explained by the well-known role of galectin-3 in inflammation and wound-healing processes. Galectin-3 is known to increase neutrophil adhesion to laminin and endothelial cells, inducing the release of mediators by mast cells, acting as a chemoattractant and increasing the production of superoxide anions for macrophages and monocytes, among others (6, 7). Surgical resection is an invasive procedure known to cause an intense inflammatory response, aiming at tissue repair, therefore it was not surprising to find - even at 1 month after the surgery - increased serum levels of galectin-3 (8).

The metastatic process is highly dependent on evasion of tumor cells from primary tumor sites and their survival in the circulation. Numerous studies suggest that galectin-3 plays a key role in tumor metastasis in vivo (9-11). Galectin-3 interaction with the ECM, such as laminin and fibronectin...
and with cell-surface proteins suggests that galectin-3 acts as a bridge between tumor cells and the ECM and other cells. Additionally, it was demonstrated that galectin-3 is a mediator of homotypic cell–cell adhesion by interacting with complementary glycoproteins and it is involved in the formation of tumor emboli and dissemination of tumor cells in the circulation; it is also involved in tumor cell survival in blood vessels by protection against anoikis.

In the present study, statistically significantly higher serum galectin-3 levels were observed from the seventh month after surgery in the female dogs of the metastatic group compared to those of the non-metastatic group. At least two explanations for this finding are suggested: a) in metastatic patients the galectin-3 level remains persistently increased postsurgery and b) the development of visible metastatic spread of CMMT is paralleled by an increasing concentration.

Figure 2. Serum galectin-3 levels for the metastatic (M) and the non-metastatic (NM) groups before the surgery (A) and at 1 (T1; B), 4 (T4; C), 7 (T7; D), 10 (T10; E), and 13 (T13; F) months after surgery.
of galectin-3 in the circulation. Regardless of how high the serum galectin-3 level is, it may favor metastasis by improving adhesive interactions between endothelial and tumor cells; promoting aggregation of tumor cell emboli by enhancing cell–cell adhesion; acting as an anti-apoptotic signal to metastatic cells; improving adhesion between the ECM and tumor cells and therefore ultimately allowing metastatic CMMT cells to reach a metastatic site and establish themselves (5, 11).

In summary, data obtained in this work suggest that the detection of increased serum galectin-3 levels in certain dogs with CMMT may reflect some biological aspects related to the metastatic process. Therefore, it is possible that an assay for detection of serum galectin-3 level is of value in monitoring follow-up of metastatic CMMT. However more studies are necessary to determine the clinical value of these results: study replication including the comparison with healthy subjects (to assess normal galectin-3 values in serum); in female dogs bearing benign tumors to avoid disparities due to tumor-induced inflammation; and finally, more female dogs with metastatic disease are required since the population involved in this study was rather small.

Conflicts of Interest

None of the Authors has a financial or personal relationship with other people or organisations that could inappropriately influence or bias the content of the article.

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References