Immunohistochemical Evaluation of the Immune Microenvironment in Patient-Matched Primary and Metastatic Canine Osteosarcoma Tumors

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Introduction

The prognosis associated with canine osteosarcoma (cOSA) has stagnated over the past 30 years, despite utilization of several different cytotoxic agents. Immunotherapeutics targeting defects in the host’s anti-tumor immune response, or promoting an enhanced preexisting response are proving effective in treating several solid human cancers previously thought to be relatively non-immunogenic. Therefore, investigation into potential immune targets in cOSA is warranted. cOSA is known to elicit a modest inflammatory response, as evident by the presence of increased circulating myeloid-derived suppressor cells (MDSCs) and elevated immune complexes in patients compared to healthy dogs. Furthermore, increased blood monocyte and lymphocyte counts, decreased blood CD8/Treg ratio, post-operative wound infection, and decreased post-treatment tumor infiltrating lymphocytes correlate with a poorer prognosis in cOSA.

Characterization of lymphocyte infiltrates within primary cOSA tumors has predominated, despite the fact that metastatic lesions remain the most common cause of cancer-related death. Moreover, immunotherapeutics such as L-MTP-PE and HER2-targeting Listeria, are thought to mediate their effects via macrophage activation. While tumor associated macrophages have commonly been found to have a positive effect on patient outcome in human OSA, this has not been evaluated in cOSA.

We therefore sought to characterize lymphocyte and macrophage infiltration in paired primary and metastatic cOSA lesions. In addition, we evaluated the prognostic significance of these infiltrates in a separate cohort of dogs treated with amputation and 6 doses of carboplatin.

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References:

Materials and Methods

Cohort 1 – Paired primary and metastatic OSA FFPE tissue samples from 21 dogs. 15 dogs were treated with amputation and later returned for euthanasia and necropsy. 5 dogs presented with disseminated disease and did not receive treatment prior to euthanasia.

Cohort 2 – 30 dogs with appendicular OSA undergoing amputation and carboplatin chemotherapy, were followed prospectively. Of these, 26 dogs had available FFPE primary tumor samples.

Immunohistochemistry (IHC) was performed to evaluate lymphocyte (CD3; CD3-12) and macrophage (CD204; SRA-E5) tumor infiltration. Quantification was performed by selecting 3 highly cellular areas from H&E stained slides, collecting 100x images of the IHC slides from each of these areas, and determining the proportion of the field taken up by positively staining cells using ImageJ. The average area across the 3 fields was used for analysis. Statistical analysis was performed with GraphPad Prism, using a Wilcoxon signed rank test for paired data, a Mann-Whitney test for comparison of 2 groups, and Kaplan Meier plots with a Log-rank test for survival analysis.

Results

Characterization of lymphocyte and macrophage infiltration in paired primary and metastatic cOSA tumors. Cohort 1; CD3 n = 18, CD204 n = 20. * = P < 0.05

Conclusions:
- Tumor infiltrating lymphocytes and macrophages tend to increase in metastatic lesions compared to primary tumors.
- Macrophages may have anti-tumor activity in cOSA.