POSTER 14

An Increased Expression of ATOX1 and Copper Levels in Canines with Non-Metastatic Osteosarcoma

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Level of Evidence: N/A (Animal Study)

Background: Osteosarcoma (OS) is responsible for a large proportion of human pediatric cancer and outcomes have not improved for patients with metastatic disease for over 30 years. As OS represents only 500-700 new cancer diagnoses/year, there is an inherent paucity of human tissue to study. Canine OS is over 20 times more prevalent than human OS and accurately reproduces clinical progression seen in pediatric patients as they exhibit a compressed pulmonary metastatic clinical course. Comparative oncology of pediatric and canine OS provides a unique opportunity to complement and accelerate translational research of metastatic OS. The concentration of intracellular copper has been a predictor of metastatic disease and antineoplastic therapies have been shown to target copper-regulating proteins in OS.

Questions/Purposes: The purposes of our study are to 1-Compare the gene expression of copper-regulating proteins in dog OS versus normal bone cells and 2- Examine differences in copper concentrations of OS, normal bone, and serum in dogs with non-metastatic disease.

Patients and Methods: This is an animal study in dogs with non-metastatic OS that underwent limb amputation surgery by a veterinary orthopedic oncology surgeon. After informed consent by the owners was obtained, serum, osteosarcoma tumor (tumor), and normal bone (bone) samples were harvested from dogs at the time of surgery. Following harvest, tumor and bone samples were stored at -80ºC and ground into a fine powder. RNA was extracted from powdered samples and gene expression was performed using quantitative polymerase chain reaction (qPCR). Serum, tumor, and bone samples were also sent for measurement of copper concentration. Statistical analysis of gene expression and copper levels were conducted through T-tests and ANOVA, respectively.

Results: Twenty-four dogs were included in the study. When compared with bone samples, tumor samples had higher levels of expression of the Antioxidant 1 copper chaperone gene (ATOX1) (**Figure 1**). For the copper concentration analysis, four values were considered outliers (Over 3 x IQR above the third quartile) and excluded from final analysis. Average copper levels of serum (505 ng/mL, SD 124.62) and bone (576 ng/mg, SD 268.72) were not statistically significant from each other ($p=0.724$). However, average copper levels found in tumor samples was 809 (SD 452.98), significantly higher than that of both Serum ($p< 0.010$) and Bone ($p=0.038$) (**Figure 2**).

Conclusion: Of the numerous genes involved in copper transport, copper-transport protein ATOX1 has been found to be the most dysregulated. It is also worth noting that copper concentrations in these tumors are significantly higher than those of normal bone or serum. ATOX1 has been shown to play a key role in regulating intracellular levels of copper[1]. Inhibition of ATOX1 has been shown to reduce cell viability in both canine and human cancer cell lines[2]. This suggests that copper dysregulation may be a key mechanism in OS cells, like that of human OS. Limitations include a low sample size. Despite this, canines may provide a readily accessible animal model to study copper dysregulation in OS. Further investigation is ongoing with other genes known to be over-expressed in OS such as VEGF and BMP4.

Figure 1: Gene expression fold change comparison in Canine osteosarcoma cells (Tumor) versus normal bone cells (Bone). Gene expression of copper chaperone gene ATOX1 is significantly greater in tumor samples than that of bone samples. Values expressed as Mean ±SEM.

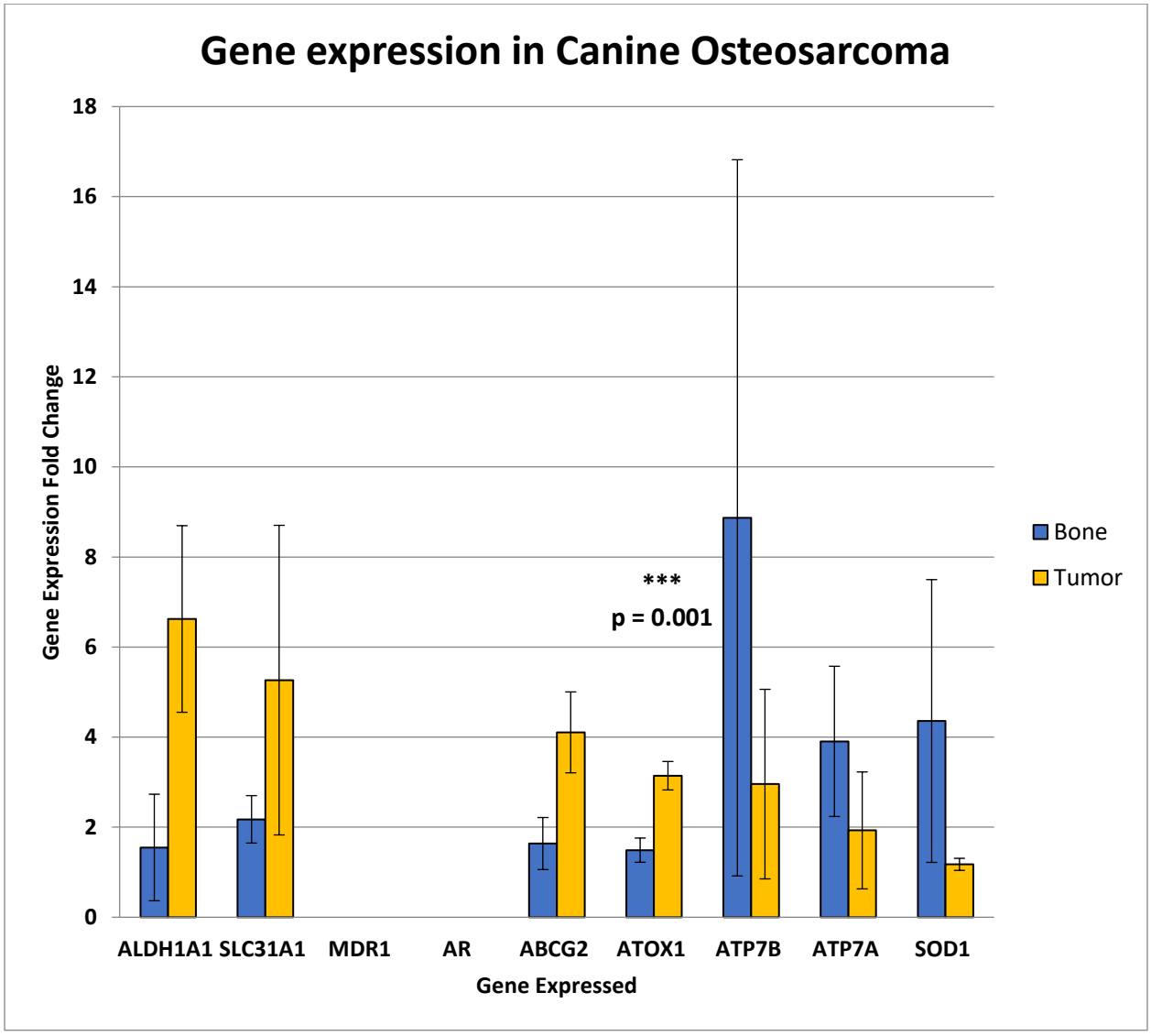

Figure 2: Average copper levels found in blood (Serum), normal bone (Bone), and osteosarcoma (tumor) samples of dogs undergoing limb amputation surgery for non-metastatic osteosarcoma. Average copper levels were significantly higher in tumor samples than they were in either serum or bone. Values expressed as mean ± SD.