Targeted Drug Delivery via Magnetic Nanoparticles: Novel Treatment for Canine Osteosarcoma

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Statement of Purpose: Osteosarcoma is the most common primary skeletal tumor found in dogs. This type of cancer is known for its aggressiveness because microscopic pulmonary metastasis typically occurs prior to owner discovery and clinical diagnosis[1]. Eighty percent of afflicted canine cases will die from tumor related complications[5]. Current therapeutic approaches are limited to amputation or limb salvage surgery[4]. Commonly, measures include adjunctive along with chemotherapy. Dogs that are more prone to develop osteosarcoma tend to be older ones, meaning they aren't physically at their best to take chemotherapy[2]. Survival rates, even with chemotherapy, are still low with around a 25% survival rate at the two year mark following treatment[1]. Recurrence is especially high for incompletely resected tumors.

This project proposes engineering a minimally invasive, targeted drug delivery system that promises a more effective treatment for canine osteosarcoma. The base of the system, as shown in figure 1, will be a magnetic nanoparticle. The nanoparticle will have attached to it a VEGF antibody and ligand CD80 that are functionalized to a biocompatible coating via emulsive polymerization. The VEGF antibody targets the antibodies associated with osteosarcoma tumor. Then, ligand CD80 induces apoptosis, or cell death, when interaction occurs with the CTLA-4 receptor on the tumor's surface[3]. This minimally invasive treatment has the potential to reduce negative side effects seen in present treatments by more efficiently targeting cancerous cells. The primary investigation of this experiment was to find the most effective concentration of proteins to reduce osteoblast proliferation. Future investigations will further investigate the most effective dose concentrations for treatment optimization *in vivo*.

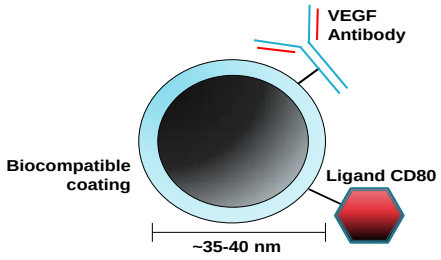

Figure 1. Schematic of a magnetic nanoparticle with biocompatible coating vectorized with VEGF antibody and Ligand CD80

Methods: The overall experiment was an exploration of protein concentrations introduced to osteoblasts in order to determine the most efficient at inducing apoptosis. A modified Ocean NanoTech Conjugation Protocol was used for conjugation of iron oxide magnetic nanoparticles to the proteins (Ocean NanoTech- 7964 Arjons Drive/ Suite G/ San Diego, CA 92126). The nanoparticles were conjugated with several concentrations of the proteins: 0.1, 1, 10, and 100 µg/ml of VEGF, ligand CD80, and both proteins. To ensure attachment of the proteins to the nanoparticles, gel electrophoresis was performed on the nanoparticle mixtures and compared to a control of just the nanoparticles. Nanoparticles were then introduced to six-well plates seeded with 2.21×10^6 ATCC CRL-2836 mouse osteoblasts over 72 hours, with one inoculation occurring every 24 hours. Cell counts were performed every 24 hours before the addition of more nanoparticles to determine the rate of cell proliferation. The nanoparticle-treated osteoblasts were compared to an untreated control group. Graphical representation of cell count data and statistical significance were determined and analyzed for all concentrations.

Results:

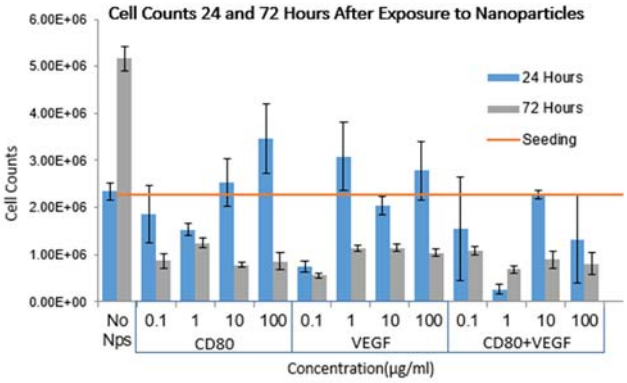

Figure 2. Graphical representation of cell counts performed on 6-well plates treated with CD80, VEGF and CD80+VEGF conjugated nanoparticles at various concentrations.

Conclusions: When comparing the different concentrations, the most effective mixture was VEGF antibody + ligand CD80 at 1 µg/ml. This was most effective at 24 hours and was the second most effective treatment for the 72 hour test. Overall, cell proliferation data also shows that multiple treatments over 72 hours were more effective at inducing apoptosis than a single initial treatment. For the 10 and 100 µg/ml concentration of CD80+VEGF, for example, cell counts were higher at the 24 hour mark, but dropped significantly at the 72 hour mark. Future studies will investigate the targeted drug delivery system in the canine model. Results support implications for the need for multiple temporal treatments in the animal model. Future work is needed to assess in vivo efficacy and dose interval.

References:

[1](Fruit, L. Indiana ADDL. 2005: Fall Newsletter. "Canine Osteosarcoma.")

[2](J Natl Cancer Inst. 1979; 62(3):537-45.)

[3](Contardi, E. Int J Cancer. 2005: 538-50.)

[4](WSU. CVM. 2009. "Cancer in Animals.")

[5](Straw, RC. Vet Clin North Am Small Anim Pract. 1990; 20(4):1141-61.)