Canine NK cell expansion and novel xenograft model for adoptive immunotherapy of osteosarcoma

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BACKGROUND

- Osteosarcoma is the most common bone cancer in both canine and human patients, but dogs develop osteosarcomas with an incidence of twenty times that of people.
- In both species, metastatic and relapsed disease have poor survival with current chemotherapy and surgical treatments.
- The usage of ex vivo activated natural killer (NK) cells as an adoptive immunotherapy is a promising approach for osteosarcoma treatment, but xenograft models of canine osteosarcoma are not yet available.

OBJECTIVES

- Develop a methodology to expand and activate canine NK cells ex vivo
- Develop an in vivo xenograft model of canine osteosarcoma using immunodeficient mice
- Demonstrate cytotoxicity of canine NK cells vs canine osteosarcoma cells in vitro

METHODS

RESULTS

Canine natural killer cells cultured with feeder cells and cytokines ex vivo show peak expansion at 14 days

CONCLUSIONS:

Canine natural killer cells can be expanded and activated using feeder cells and cytokines readily available in human NK cell trials and show potent cytotoxicity against osteosarcoma in vitro. The canine D17 mKate2 xenograft model recapitulates human osteosarcoma growth locally and metastasizes, making it a viable platform for testing adoptive immunotherapy with NK cells.

Canine osteosarcoma cells can be detected via immunofluorescence in tissue samples

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Canine osteosarcoma shows bony tumor formation as well as lung and bone metastasis in a xenograft model

Fluorescent images were taken via IVIS on day 90 post injections of D17 mKate2 canine osteosarcoma cells. A. Lateral view of mice receiving right flank subcutaneous injections of 1E6 D17 mKate2 cells versus control. B. Anteroposterior view of mouse receiving tail vein IV injection of 5E6 D17mKate2 cells versus control.

NG6 mice were injected intravenously with 5E6 D17 mKate2 osteosarcoma cells. Faxitron images were taken on day 90 of representative mouse. A. Posteroanterior view, red circles show tumor formation in left tibia and bony destruction of spine. B. Lateral view, red circles show lung metastases in the same animal.

Cytotoxicity of effector canine NK cells vs target canine osteosarcoma (d17 mKate2) cells using Luminex. Cells were plated at E:T ratios of 1.1:50. A, overlap counts of target mKate2 and caspase-3 488nm signals over 22 hours. B, Overlay count at 24 hours.