Canine osteosarcoma as a model for investigating the therapeutic potential of miR-34a prodrug in humans

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INTRODUCTION

Osteosarcoma (OS) is the most common primary malignant bone tumor seen in both children and dogs, with incidence in dogs estimated to be more than ten times greater than in humans [1]. There are many shared features between human and canine OS, making dogs a valuable disease model to study [2, 3]. Currently, standard of care treatment for both humans and dogs is a combination of surgery and chemotherapy [2, 4]. Survival times have not improved within the last fifteen years, with 30-40% of children and >90% of dogs ultimately dying from OS [1]. The aggressive and incurable nature of this disease highlights the need for novel treatment modalities.

RESULTS

Figure 1. Canine OS cells were transfected with increasing concentrations of miR-34a produg or tRNA/MSA prior to measurement of viable cells using MTT assay. Marked reductions in proliferation are observed in Abrams (A) and HMPOS (B) canine OS cell lines with miR-34a produg. Moderate antiproliferative effects were seen following transfection with tRNA/MSA.

Figure 2. (A) Representative image of Abrams OS cells transfected with 10 nM miR-34a produg and incubated for 72 hours prior to replating for clonogenic assay. (B) Quantification of surviving fraction of canine OS cells following transfection with 10 nM miR-34a produg demonstrates a significant reduction in colony forming ability for Abrams OS cells (unpaired T-test with Welch's correction for unequal variance).

Figure 3. Left: Quantitative real-time PCR of canine OS cells transfected with miR-34a produg or control compound. Results demonstrate increased expression of mature miR-34a, verifying the processing of produg into mature miR-34a. Right: structure of the tRNA/miR-34a produg and the mature miR measured by qRT-PCR.

CONCLUSIONS AND FURTHER DIRECTION

• The bioengineered produg is processed into the active form of miR-34a intracellularly in canine OS cell lines and functions to decrease levels of target proteins, similar to results found in human OS cell lines.
• Through its regulatory actions on target proteins, miR-34a decreases proliferation in canine OS cells and decreases clonogenic ability as well.
• Additional proliferative and clonogenic testing is required to assess the effects of the produg and tRNA/MSA with a larger range of concentrations.
• Future experiments include investigations into the ability of the produg to induce apoptosis and assessment of the effects on migration and invasion of tumor cells.

MATERIALS AND METHODS

Cell Viability Assays:

Proliferation – HMPOS and Abrams canine OS cell lines were transfected with miR-34a produg or MSA (control tRNA scaffold) using Lipofectamine® 3000 or jetPRIME® for 8 hours. Media was replaced and cells were incubated for another 48 hours; relative viable cell number was assessed via a bioreductive assay using MTT.

Clonogenic – OS cells were transfected in the same manner as for proliferation; following 72 hour incubation, single cell suspensions were plated and grown for 7 days. Colonies were fixed and stained with crystal violet before counting.

Demonstration of target modulation:

Western blot – OS cells were transfected overnight with 10 nM miR-34a produg prior to protein extraction and analysis of miR-34a target proteins.

Demonstration of intracellular produg processing:

Quantitative real-time PCR – OS cells were transfected with 10 nM miR-34a produg or tRNA/MSA; total RNA was isolated using TRIzol® and reverse transcribed with a miR-34a specific primer. qRT-PCR was performed with RP55 as a housekeeping gene. Relative expression was determined via the 2^ΔΔCt method.

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REFERENCES


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