Materials and Methods

Cell Lines

Three canine OS cell lines including HMPOS (provided by Dr. James Farese, University of Florida), Abrams (provided by Dr. Douglas Thamm, Colorado State University), and K003 (provided by Dr. Chand Khanna, National Cancer Institute) were utilized in this study. Cells were cultured at 37˚C in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with glutamine (2 mmol/l), penicillin (100 IU/ml), streptomycin (100 IU/ml), and 10% fetal bovine serum (FBS) in a humidified atmosphere supplemented with 5% CO_2.

Real-Time Polymerase Chain Reaction

RNA was collected from the cultured cell lines using the RNeasy minikit by Qiagen. RNA was treated with Turbo DNAse from Life Technologies to remove excess DNA. The concentration and purity of the RNA samples were determined using the NanoDrop2000c. Four microliters of each RNA sample was then added to the SYBR Green PCR MasterMix by ThermoFisher Scientific with the appropriate quantitative primers for CXCR4. Reactions were performed in a ABI 700 Real-Time PCR System by Biolife Systems with the following conditions: 15 minutes at 37.0°C for Reverse Transcription, 10 minutes at 95.0°C for reverse transcription activation, 40 cycles (95.0°C for 10 seconds, 60.0°C for 30 seconds, 72.0°C for 30 seconds) and concluded by 95.0°C for 10 minutes for dissociation.

Primer Sequences

CXCR4: Forward-TCTGTGGCAGACCTCCTCTT
CXCR4: Reverse-TGAAACTGGAACACCACCAA
GAPDH: Forward-CTGTGGGCAAGGTCATCC
GAPDH: Reverse-GAAGCCCATGCCAGTGAG