Introduction

Osteosarcoma (OS) is the most common primary bone tumor found in children and young adults.[1] We have previously shown that alpha-CaMKII (Ca2+/calmodulin kinase 2) (α-CaMKII) regulates vascular endothelial growth factor (VEGF) and its autocrine signaling functions in human OS.[2]

Disease recurrence is a major challenge in the clinical management of OS and is thought to be caused by a small subpopulation of tumor-initiating stem cells (TISCs).[3]

Human OS TISCs are characterized by their expression of CD117* and Sca-1*, and the stem cell transcription factors Sox2, Nanog and Oct4.[4]

These TISCs are seen to have high levels of VEGF factor in a variety of cancers.[5,6,7]

Hypothesis

Tumor-initiating stem cells are regulated by α-CaMKII and VEGF in human osteosarcoma

Methods

Cell Culture: 143B human osteosarcoma cells were purchased from American Type Culture Collection (ATCC, Manassas, VA, USA). Cells were maintained in DMEM supplemented with 10% FBS, 1% penicillin/streptomycin at 37°C with 5% CO2.

RNA Extraction and RT-PCR: Total RNA was extracted using the Trizol method as recommended by the manufacturer (Invitrogen). One μg of RNA was reverse-transcribed using M-MuLV reverse transcriptase, and the equivalent of 10 ng was used for Syber Green real-time quantitative RT-PCR.

Western Blot-ER: Cells were harvested for protein extraction using a lysis buffer containing 30mM Tris pH 7.4, 150mM NaCl, 1% IGEPAL CA-630 and 10% glycerol. Extracts were loaded (20μg/lane) on a 10% mini-Page system and transferred to a PVDF membrane. After blocking, the membrane was incubated with the indicated antibodies. Signals were detected using a peroxidase-conjugated secondary antibody and an enhanced chemiluminescence detection kit (ECL,Amersham Biosciences).

Enzyme-linked immunosorbent assay (ELISA): A VEGF sandwich ELISA kit (Invitrogen) for detecting human VEGF165 in osteosarcoma cell-conditioned media was used. A polyclonal antibody specific for human VEGF was coated onto microtiter strips. Samples and standards were pipetted into these wells. During the first incubation, the human VEGF antigen binds to the immobilized antibody. After washing, a biotinylated monoclonal antibody specific for human VEGF was added. During the second incubation, this antibody binds to the immobilized antibody. After washing, a horseradish peroxidase-conjugated secondary antibody and an enhanced chemiluminescence detection kit (ECL) were added to the wells. The plates were read using the luminometry reader.

Immunohistochemistry: Tissues were deparaffinized and rehydrated followed by antigen retrieval using 10mM sodium citrate buffer, pH 6. Samples were blocked for 1 hour in 5% goat serum, treated with a 1:100 dilution of biotinylated antibody specific for α-CaMKII or anti-human VEGF and stained using a horseradish peroxidase-conjugated secondary antibody and an enhanced chemiluminescence detection kit (ECL). The tissues were counterstained with hematoxylin for 30 seconds.

Results

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Figure 1. Treatment with Tamoxifen and/or Avastin decreases α-CaMKII and VEGF in 143B osteosarcoma cells

Figure 2. Treatment with Tamoxifen and/or Avastin decreases Nanog, 4-Oct and Sox2 gene expression and protein levels in 143B osteosarcoma cells

Figure 3. Treatment with Tamoxifen and/or Avastin decreases Nanog, 4-Oct and Sox2 gene expression and protein levels in 143B osteosarcoma cells

Figure 4. Treatment with Tamoxifen and/or Avastin decreases CD117 and Sca-1 osteosarcoma tumor-initiating stem cells

Conclusion

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References


