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TITLE
The small molecule inhibitor KGP-94 attenuates Cathepsin L and K mediated tumor angiogenesis

HYPOTHESIS:
We hypothesize that inhibition of the proteolytic enzymes Cathepsin L (CTSL) and Cathepsin K (CTSK) will impair osteosarcoma (OS) metastasis.

BACKGROUND/AIMS:
Osteosarcoma (OS) is the most common malignant bone cancer, primarily affecting patient in late adolescence and young adulthood. Currently, OS is the third leading cause of cancer related deaths within the pediatric population. At diagnosis, approximately 90% of patients present with clinical or subclinical metastases. Tumor growth and metastasis is driven by angiogenesis. In OS, cysteine proteases, Cathepsin L (CTSL) and Cathepsin K (CTSK) are secreted by OS cells and promote tumor angiogenesis. Preclinical studies demonstrate that inhibition of angiogenesis significantly reduces OS metastasis. The present study examines the contribution of CTSL and CTSK to osteosarcoma angiogenesis and evaluates the anti-angiogenic efficacy of CTSL/CTSK inhibitor KGP-94.

METHODS:
Secreted CTSL/CTSK levels from human OS cells were determined by performing an ELISA on cell-conditioned media. To evaluate angiogenesis in-vitro, $7 \times 10^4$ human microvascular endothelial cells (HMVEC-L) were seeded onto 200 µL of solidified matrigel in a 24 well dish. Indicated concentrations of purified human recombinant CTSL or CTSK and KGP-94, were added to each well and incubated at 37 °C for 24 hr. Cell-conditioned media samples from this assay, were used to evaluate the role of CTSL, CTSK, and KGP-94 on angiogenic growth factors and cytokines using an angiogenesis proteome profiler array kit. The ability of KGP-94 to inhibit OS mediated angiogenesis in-vivo was evaluated using an intradermal injection. For this assay, $5 \times 10^5$ human osteosarcoma (HU09-M112) cells were injected on the ventral surface of mice at four sites. KGP-94 (10 or 20mg/kg) was administered intra-peritoneally for 3 days. The number of tumor-induced blood vessels was determined using a dissecting microscope.

RESULTS & CONCLUSIONS:
Our results demonstrate that CTSL and CTSK induce key pro-angiogenic cytokines and growth factors. Small molecule inhibition by KGP-94 overcomes increased angiogenesis induced by CTSL and CTSK in vitro and OS cell induced angiogenesis in vivo. Targeting CTSL/CTSK may provide a novel therapeutic approach for OS patients.