Excess TGF-β Induces MMP-9 and SPP-1 Expression in the Skeletal Muscle of Cancer Patients with Bone Metastases: Association with Muscle Dysfunction

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Background

Patients with bone metastases experience muscle weakness which can lead to a severe loss of muscle mass and function known as cachexia, a paraneoplastic syndrome for which there is no treatment. Bone metastases causes pathologic fractures and further increase mortality rate by 32%. Tumors in the bone lead to excessive bone resorption, releasing growth factors including TGF-β from the bone matrix. MMP-9 and SPP-1 are secreted proteins downstream in the TGF-β signaling pathway and promote the expression and activation of TGF-β. MMP-9 and SPP-1 further promote osteoclast activity and bone resorption. As muscle is one of the organ systems responsive to bone-derived signals, recent evidence suggests that pathologic, accelerated bone resorption causes muscle weakness. Muscle weakness promotes immobilization of patients, which further increases bone loss and increases fracture risk.

Clinical Problem

With the exception of treatments to reduce pain and other symptoms, bone metastases currently cannot be cured. Realizing the changes made to bone-muscle cross talk due to osteolytic bone metastasis has implications for treatment strategies and the possible identification of a gene or protein to target for intervention. However, the relationship between biochemical signals underlying this cross talk is not yet fully understood.

Hypothesis

TGF-β, MMP-9, and SPP-1 interact and fuel a feed-forward vicious cycle of bone destruction and muscle weakness as a result of osteolytic bone metastases.

Methods

We analyzed muscle biopsies and blood samples from lung, breast and renal cancer patients with bone metastases. In RNA-seq data, we observed that MMP9 and SPP1 are highly abundant in the muscle from patients with bone metastases when compared to non-tumor controls. We further validated our expression profile studies at mRNA and protein levels via western blot, ELISA and qRT-PCR. Additionally, the clinical results were confirmed by using Camurati-Engelman Disease (CED) mice with excessive TGF-β release and extreme bone turnover.

Results

Plasmatic levels of MMP-9 and SPP1 in patients with bone metastases. We further analyzed muscle biopsies and blood samples from breast cancer patients with bone metastases. We compared to non-tumor controls. We further validated our expression profile studies at mRNA and protein levels via western blot, ELISA and qRT-PCR. Additionally, the clinical results were confirmed by using Camurati-Engelman Disease (CED) mice with excessive TGF-β release and extreme bone turnover.

Animal Results

CED (Camurati-Engelman Disease) TGF-β Mouse Model

Analysis of Grip Strength in CED Mice Compared to Wild Type

Figure 7: CED is a skeletal condition characterized by abnormally thick bones due to a mutation in the TGF-β1 gene. Normally, the TGF-β protein aids in maintaining bone mass by coupling bone resorption and formation. The CED mutation, however, results in the production of overly active TGF-β proteins, increasing bone formation.

Figure 8: Four paw grip strength test indicates decreases in muscle strength of CED mice compared to wild type controls.

Figure 10: CED mice display a decrease in bone mineral density as quantified by DXA when compared to age-matched wild type controls, indicating weaker bones.

Figure 11: Western blot of Mmp9 and Spp1 in the quadriceps muscle of 4-month-old WT and CED mice. Quantification of plasmatic Mmp9 and Spp1 expression.