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

Isabella Ya-Zhi Hendricks[1], Lei Shi[1], Sukanya Suresh[1], Truc Kuo[1], Xu Cao[2], Theresa A Guise[1]

[1] Department of Endocrine Neoplasia and HD, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA;
[2] Department of Department of Orthopedic Surgery, The Johns Hopkins School of Medicine, Baltimore, MD 21205, USA.

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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-Engelmann Disease (CED) mice with excessive TGF-β release and extreme bone turnover.

Clinical Results

Myofiber Cross Section Area Quantified Using Bioquant

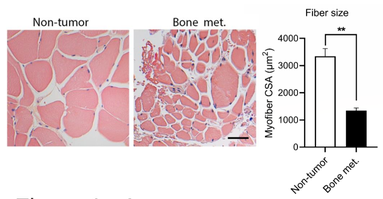


Figure 1: A representative photomicrograph of H&E staining of skeletal muscle section. Quantitative estimation of myofibers fiber cross-sectional area of quadriceps muscle.

Upregulated Gene in Muscle from Patients with Bone Metastases

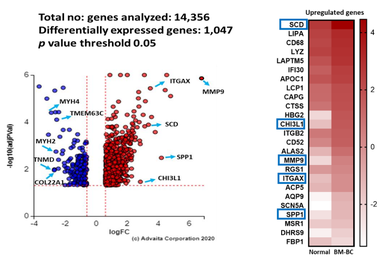


Figure 4: Gene expression of MMP-9 and SPP-1 were significantly increased in the skeletal muscle from patients with bone metastases.

IHC Staining of p-Smad2/3

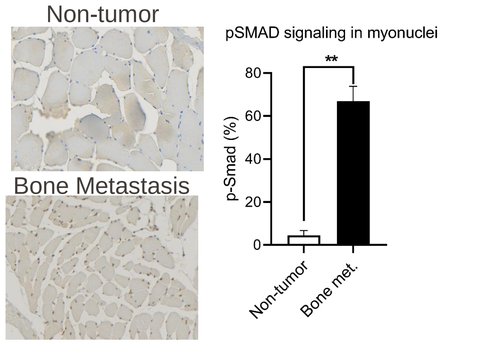


Figure 2: IHC (immunohistochemical) staining for phospho-Smad2 and 3 (p-Smad2/3) in the muscle biopsies from breast cancer patients with bone metastasis (bottom left) and non-tumor controls (top left). Quantification of the percentage of p-Smad2/3 positive myonuclei (right).

Level of Expression of MMP-9 and SPP-1 mRNA and Proteins

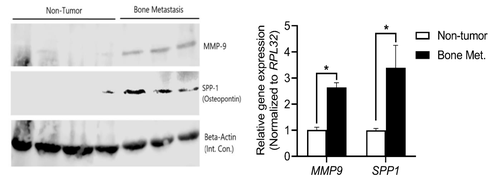


Figure 5: Western blot (left) and real-time RT-PCR analysis (right) of muscle biopsies from breast cancer patients with bone metastases indicating heightened expression

TGF-β1 Concentration in Platelet-Free Plasma

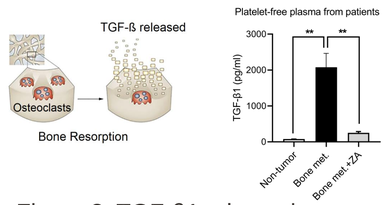


Figure 3: TGF-β1 release is quantified using ELISA. Concentration is elevated in the platelet-free plasma of patients with bone metastases as compared to non-tumor controls and reduced with bisphosphonate treatment.

Plasmatic levels of MMP-9 and SSP-1

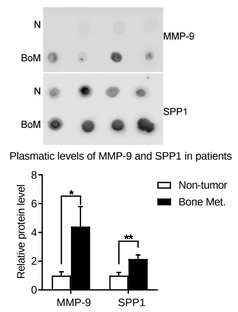


Figure 6: Dot blots of plasmatic expression of MMP-9 and SPP1 expression. Quantification of dot blot bands from breast cancer patients with bone metastases and controls

Animal Results

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

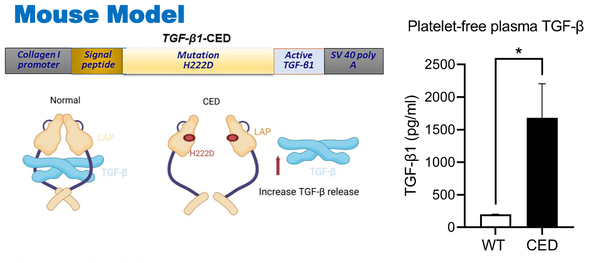


Figure 7: CED is a skeletal condition characterized by abnormally thick bones due to a mutation in the TGF-β1 gene. Normally, the TGF-β protein aids in maintaining bone mass by coupling bone resorption and formation. The CED mutation however, results in the production of overly active TGF-β proteins,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

Analysis of Grip Strength in CED Mice Compared to Wild Type

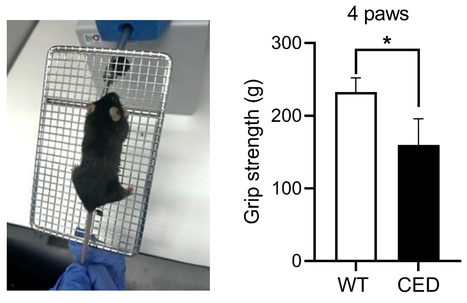


Figure 8: Four paw grip strength test indicates decreases in muscle strength of CED mice compared to wild type controls.

Right Hind Leg X-ray of CED and WT Mice

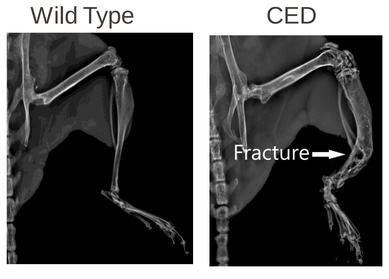


Figure 9: CED mice display significant bone damage and mutation as compared to WT controls.

Total Bone Mineral Density in CED Mice as Compared to Age-matched WT Controls

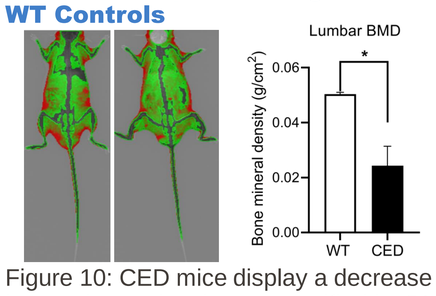


Figure 10: CED mice display a decrease in bone mineral density as quantified by DXA when compared to age-matched wild type controls, indicating weaker bones.

Level of Expression of MMP-9 and SPP-1 in Mouse Samples

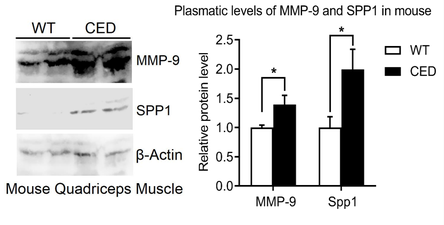
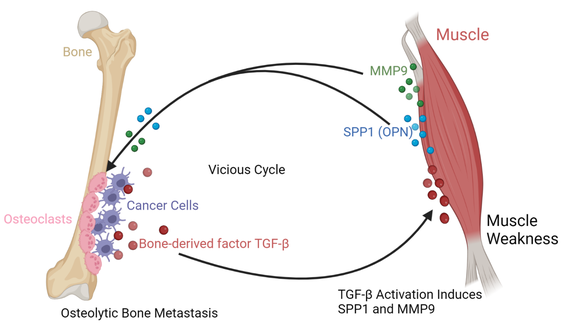


Figure 11: Western blot of Mmp9 and Spp1 in the quadriceps muscle of 4-month-old WT and CED mice. Quantification of plasmatic Mmp9 and Spp1 expression.

Conclusions

Results indicate that pre-cachexia secretion driven by cancer-related bone loss is a central mediator of muscle tissue breakdown. TGF-β, MMP-9, and SPP-1 concentrations are elevated in the muscle biopsies and plasma of patients with bone metastases and CED mouse models. Additionally, muscle mass in patients and muscle strength in CED mice is reduced. Both MMP-9 and SPP-1 can be induced by TGF-β cytokines while the secreted proteins are additionally capable of promoting TGF-β and osteoclast activation. We found that bone-derived TGF-β and muscle-mediated secretion of MMP-9 and SPP-1 fuel a feed-forward vicious cycle of muscle weakness and bone destruction. This provides insight into the mechanism of bone-muscle cross talk in patients with osteolytic bone metastases. Our findings reinforce the idea that factors driven by cancer-related bone loss is a central mediator of tissue breakdown. In the future, we propose to alleviate bone metastases-associated muscle weakness and test antiresorptive medication and Low-intensity Vibrations (LiV) therapy as interventions. LiV uses Low-intensity Vibrations to gently stimulate the body. These precise movements encourage the body's bone-building cells (osteoblasts) to work, which is a safe and natural way to improve bone health that has been scientifically researched to work.



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2 Tang & Xu, *Nat Med*. 2009 Jul; 15(7):757-65.

Acknowledgments

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