Ablation of Tumor Specific IGFBP-3 Attenuates PDAC-Induced Skeletal Muscle Wasting and Reduces Markers Associated with Severe Disease

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INTRODUCTION: Pancreatic ductal adenocarcinoma (PDAC) is the fourth leading cause of cancer-related deaths, and its incidence is expected to rise. Importantly, a large percentage of patients with PDAC experience cancer-related SMW, which is a growing burden among cancer survivors. As such, SMW is prognostic of treatment failure, radiotherapy toxicity, and a shorter time to tumor progression related to survival. Furthermore, the roles of TGF-β signaling, and ubiquitin proteasome pathway (UPP) activation have been duly noted in SMW. However, their role in PDAC-related SMW has yet to be elucidated. Recent literature supports an interaction between IGFBP-3 and TGF-β signaling in muscle. Lastly, findings from our lab highlight the relationship among immune cell infiltration IGFBP-3 upregulation, and SMW in a murine model of PDAC. Here we hypothesized that genetic depletion of IGFBP-3 in the KCKO PDAC tumor cell line decreases its ability to induce SMW in mice via TGF-β signaling in skeletal muscle and cellular infiltration in a model of PDAC-related SMW.

METHODS: All experiments were approved by the University Committee on Animal Resources and were performed in compliance with the National Institutes of Health (NIH) and University of Rochester-approved guidelines for the care and use of animals. Before tumor inoculation, C57BL/6J female mice (6-8 weeks old) were scanned using dual energy X-ray absorptiometry (DEXA) to acquire baseline lean mass measurements of the lower hindlimbs. Mice were randomized into two experimental tumor-bearing groups based on starting lean mass and body weight (n=14/group): 1) parental KCKO-Luc (PDAC), or 2) KCKO-Luc IGFBP-3−/− (KO). Tumor-bearing mice were compared to healthy non-tumor control (NTC) (n=10) littermates. The mice were injected orthotopically with 1x106 of either murine KCKO-Luc or IGFBP-3 KO tumor cells in 0.1 mL of Matrigel solution. In vivo bioluminescence imaging was performed on day 7, post tumor cell inoculation, to verify tumor engraftment. Weekly DEXA scans monitored longitudinal changes in lean mass. Mice were monitored and sacrificed based on previously established failure to thrive criteria or at the predetermined 100-day endpoint. At sacrifice, serum, and legs were harvested. Quadriiceps and tibialis anterior (TA), muscles were preserved for transcriptional analysis and histology. ELISA was performed on serum for the quantification of IGFBP-3. RNA was extracted from quadriiceps muscles for transcriptional analysis of igfbp3, tgfβr1, UPP associated genes: trim63 and fbxo32, and macrophage activation marker cebpb. TA muscles were labeled with anti-fibronectin antibodies and DAPI to identify nuclei/tissue area and quantified via Visiopharm analysis. Statistical analyses were performed using GraphPad Prism software. One-way ANOVA was used to analyze within group and between group changes in IGFBP-3 concentrations, lean mass, transcriptional expression, and nuclear number. Kaplan-Meier estimator of survival was used to quantify survival of experimental mice. p < 0.05 was considered significant (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

RESULTS: PDAC mice experience significantly reduced survival compared to KO mice that maintained 100% survival at day 100 (p<0.0001) (Fig. 1A). Reductions in PDAC survival correspond with increases in systemic IGFBP-3 compared to NTC (p<0.01) and KO mice (p < 0.001) while no difference was observed between NTC and KO animals (Fig. 1B). Furthermore, PDAC mice lost significantly more lean mass as measured by DEXA compared to NTC mice (p<0.0001). KO mice experienced a significant loss of lean mass compared to NTC mice (p<0.05), however, they also experienced a significant attenuation when compared to PDAC mice (p>0.0001) (Fig. 2A). Representative DEXA images confirm relative differences in muscle mass and size (Fig. 2B-D). RT-qPCR analysis on muscle indicated that PDAC mice display significantly increased expression of igfbp3 and UPP associated genes trim63 and fbxo32 compared to NTC and KO mice while no difference was seen between NTC and KO mice. (Fig. 2E-G). Moreover, KO mice have reduced expression of tgfbr1 compared to NTC and PDAC mice (Fig. 2H), which suggests decreased TGF-β dependent catabolism in KO skeletal muscle. Lastly, PDAC muscle displays a significant increase in nuclear number when compared to NTC (p<0.05) and KO mice (p<0.01) indicative of reduced muscle health (Fig. 3A-D). Moreover, increased cellular infiltration is supported by significantly increased expression of cebpb in PDAC muscle, a known marker of macrophage activation (Fig. 3E).

DISCUSSION: In this study, we demonstrated a relationship among upregulation of IGFBP-3, TGF-β dependent UPP activation, and SMW. Tumor-specific ablation of IGFBP-3 significantly attenuates the loss of lean mass measured via DEXA and improves muscle health as indicated by reduced immune cell infiltration. Further work will validate IGFBP-3 dependent effects on SMW and investigate therapeutic neutralization of IGFBP-3 as a treatment for PDAC-induced SMW.

CLINICAL SIGNIFICANCE: SMW is a crippling co-morbidity associated with pancreatic cancer. Its diagnosis often means reduced survival, increased hospital stays, and a reduction in available treatment options. Identifying underlying mechanisms remains necessary to develop better treatment modalities that can increase patient quality of life and survival.


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