

Impaired adaptation to negative energy balance in pancreatic cancer-associated wasting

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Objective

The disease associated wasting condition, cachexia, is a common complication of pancreatic ductal adenocarcinoma (PDAC) that impacts quality of life and portends poor survival. Undernutrition is a major driver of wasting in PDAC, yet cachexia remains refractory to nutritional supplementation. By modifying nutritional challenges at different stages of cachexia development, we sought to understand the relative contributions of undernutrition and metabolic reprogramming to adipose and skeletal muscle wasting.

Hypothesis

PDAC impairs the adaptive response to metabolic stressors, leaving the host vulnerable to wasting in the context of negative energy balance

Methods

- Adult $\sqrt[3]{8}$ $\stackrel{\circ}{2}$ C57BL/6J or *II6^{-/-}* mice received orthotopic PDAC tumor (from Kras^{G12D}; p53^{R172H/+}; Pdx1-cre mice) or sham injections.
- Mice were metabolically challenged by 50% food restriction (FR) beginning 3 days after orthotopic tumor injections using 2x2 factorial study design
- Adipose and muscle mass were quantified using serial whole animal NMR and raw inguinal fat pad and gastrocnemius weight at time of necropsy
- Blood glucose and ketones were measured using point-ofcare glucometer and ketometer, respectively
- Ketogenic potential was evaluated by fasting mice overnight, followed by octanoate challenge (0.2 mg/kg)
- Liver metabolic gene expression measured using qPCR
- Food absorption (exocrine function) estimated by measuring fecal protease activity
- Statistical Analysis
 - NMR over time one way ANOVA
 - Correlations between food intake and wasting Linear regression
 - Food intake and ketone release repeated measures ANOVA
 - 2x2 factorial comparisons two way ANOVA
 - Student's t-test used to compare 2 groups
 - *, p<.05; **, p<.01; ***, p<.001





Figure 4. IL-6 ablation ameliorates but does not ablate PDAC-associated muscle wasting and hypoglycemia. (A) Experiment design. Tumor growth was not different between WT and *II6^{-/-}* mice (B). The decrease in food intake caused by PDAC was unaffected by II6 ablation (not shown). Fat mass was decreased in PDAC bearing mice, regardless of II6 presence (C). Gastrocnemius mass (D) and blood glucose (E) were decreased in PDAC bearing mice, but independently increased in *II6^{-/-}* mice. 2-way ANOVA (B-E).

Results

- Orthotopic PDAC tumors elicit progressive anorexia, fat wasting, and muscle wasting over time.
- Loss of fat mass is closely correlated to food intake (r=0.6, p<.01), whereas muscle loss was not (r=0.2, p=0.54)
- Fecal protease activity is unimpacted by orthotopic PDAC tumors
- Applying subchronic food restriction elicited equivalent adipose loss in both PDAC and sham mice, but muscle loss uniquely in PDAC mice (FR x PDAC $p_{interaction} < .05$).
- Because adaptation to metabolic stress is mediated largely by the liver, we looked at macronutrient partitioning and hepatic metabolic gene expression. Serum glucose is reduced by FR and PDAC (*p*<.05 for each). Ketogenic
- potential in fasting mice is reduced by PDAC (p<.05).
- Hepatic expression of glycolytic genes is increased by PDAC, whereas gluconeogenic and ketogenic gene expression is reduced.
- Whole body knockout of *II6* does not impair growth of PDAC.
- Whereas *II6* knockout does not impact PDAC-associated fat loss, loss of IL-6 increases muscle mass and may ameliorate PDAC-associated muscle wasting (*II6* x PDAC $p_{interaction}$ =.06) and hypoglycemia (*II6* x PDAC $p_{interaction}$ =.08)

Conclusions

- susceptibility
- 2. Metabolic reprogramming evident *early* in PDAC growth



1. Fat loss in PDAC is a function of nutrition alone, whereas muscle loss is a function of both undernutrition and *increased metabolic*

3. PDAC impairs normal hepatic adaptive responses to metabolic stress, which may explain increased vulnerability to undernutrition 4. IL-6 may mediate some of PDAC's metabolic effects on the liver