Pancreatic Tumor Burden Elicits Progressive Respiratory Muscle Pathology and Dysfunction

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ABSTRACT

Cancer cachexia is a multifaceted condition affecting a large majority of cancer patients, with pancreatic ductal adenocarcinoma (PDAC) patients presenting the highest prevalence. Cachexia is characterized by progressive whole-body and skeletal muscle wasting, including vital cardiorespiratory muscles. There are currently no medical treatments for cachexia, largely due to a limited understanding of the underlying mechanisms leading to the progression of the disease, especially with respect to cardiorespiratory muscles. The purpose of this study was therefore to characterize the time course of diaphragm muscle pathology and dysfunction in a preclinical model of PDAC. C57Bl6/J mice (male, 10 weeks old) received orthotopic injections into the pancreas of either PBS (SHAM, n=6) or mouse pancreatic cancer cells (KPC, n=30). Mice were monitored daily and euthanized at predetermined time points corresponding to 8 (D8), 10 (D10), 12 (D12), and 14 (D14) days post cancer cell inoculation or when mice met IACUC-mandated tumor endpoint (i.e. (i.e. 15-17 days post tumor cell inoculation (END), body condition score < 2). In-vivo diaphragm function was assessed via M-mode ultrasonography immediately prior to euthanasia. Hemispheres of the diaphragm were harvested and subjected to immunohistochemical analyses. In-vivo diaphragm (excursion amplitude) and respiratory function (respiratory rate, minute ventilation) were significantly impaired beginning at D12 versus Sham. Additionally, significant diaphragm muscle fiber atrophy occurred at D12, D14, and END. Collagen remodeling, indicated by collagen hybridizing peptide reactivity, was increased by 113% at D12 versus Sham. Muscle area occupied by extracellular matrix was significantly increased by 52% D12 and persisted through END. Infiltration of CD45+ leukocytes significantly increased versus Sham at D8 and steadily increased through END. The abundance of infiltrating leukocytes significantly correlated to minute ventilation, muscle fiber size, collagen remodeling, and muscle area occupied by extracellular matrix. Overall, this is the first study to characterize the time course of cachexia development and progression in the orthotopic KPC model. The current findings demonstrate an early and profound pathological remodeling of the diaphragm in response to pancreatic tumor burden, characterized by muscle fiber atrophy, extracellular matrix remodeling, immune cell infiltration, and ultimately respiratory dysfunction. These data help define the early cellular events underlying the development of muscle pathology and the associated respiratory dysfunction, providing an important translation tool for therapeutic investigations.

BACKGROUND

- Cachexia is a devastating consequence of cancer, characterized by whole-body, fat, and skeletal muscle wasting
- Cachexia is most prevalent in patients diagnosed with pancreatic ductal adenocarcinoma (PDAC)
- The loss of skeletal muscle impacts both limb muscles and cardiorespiratory muscles, which may mediate respiratory dysfunction commonly reported in patients with advanced cancers
- No approved treatments for cachexia currently exist, partly due to a limited mechanistic understanding of its etiology

OBJECTIVE

Characterize the time course of diaphragm muscle pathology and dysfunction in a preclinical, orthotopic model of pancreatic cancer

METHODOLOGY

- C57Bl6/J mice (10 weeks old, male) were randomly assigned to receive an orthotopic injection into the pancreas of either
  - Sterile PBS (SHAM) or
  - Pancreatic cancer cells (KPC), originally derived from pancreatic tumors of Kras(G12D);Trp53(R172H);Pdx1-Cre (KPC) mice
- KPC: 2.5 x 10^5 KPC cells in 50 µl PBS; SHAM: 50 µl PBS
- Mice were monitored daily until predetermined time points or until IACUC-mandated endpoint
- Skeletal muscle and tumor were dissected upon euthanasia

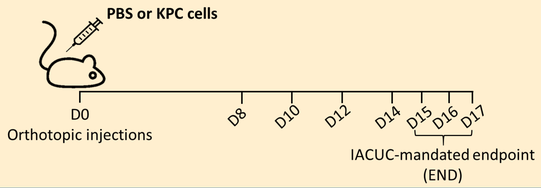

Fig.1 Schematic of the experimental design. D0-17 = 0-17 post KPC cell injections.

DEVELOPMENT OF CACHEXIA

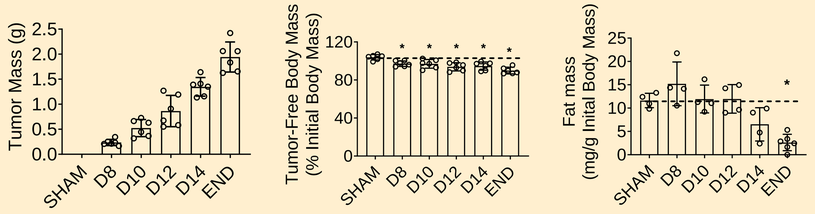

Fig.2 Body and fat wasting progression in response to tumor-burden. D8-14 = 8-14 post KPC cell injections. * = different from Sham.

IN-VIVO DIAPHRAGM FUNCTION

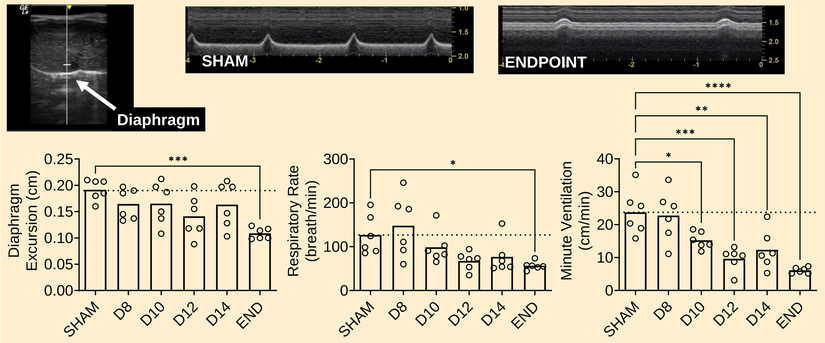

Fig.3 *In-vivo* diaphragm function, assessed via m-mode ultrasonography, is progressively impaired in response to pancreatic tumor burden. Minute ventilation = diaphragm excursion x respiratory rate. D8-14 = 8-14 post-KPC cell inoculation. *P<0.05, **P<0.01, ***P<0.001.

SKELETAL MUSCLE MORPHOLOGY

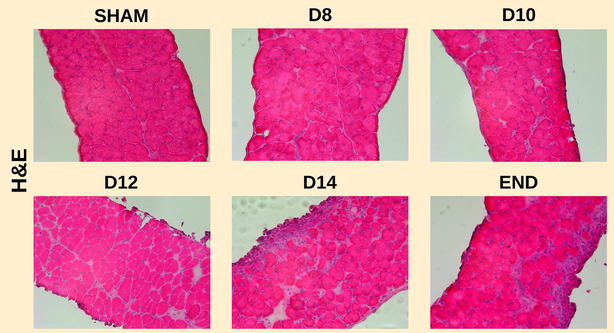

Fig.4 Hematoxylin and Eosin (H&E) stained cross sections of diaphragm (DIA) show gross morphology in SHAM and tumor-bearing (D8-END) mice. Over the course of tumor-burden, DIA exhibits increased muscle fiber atrophy, expansion of the extracellular space, and mononuclear cell infiltration. D8-14 = 8-14 post-KPC cell inoculation.

MUSCLE FIBER ATROPHY AND TYPOLOGY

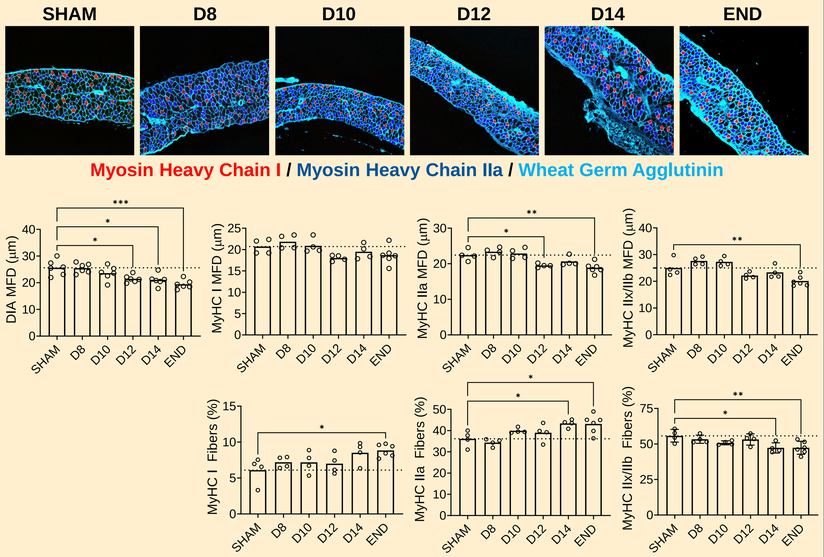

Fig.5 Diaphragm (DIA) was analyzed for changes in muscle fiber size and typology in response to pancreatic tumor-burden. Fiber size was significantly reduced by D12 and fiber typology significantly shifted by D14. Red = Myosin heavy chain (MyHC) I; Blue = MyHC IIa; Black = MyHC IIx/IIb; MFD = Minimum Feret Diameter. D8-14 = 8-14 post-KPC cell inoculation. *P<0.05, **P<0.01, ***P<0.001.

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EXTRACELLULAR MATRIX REMODELING

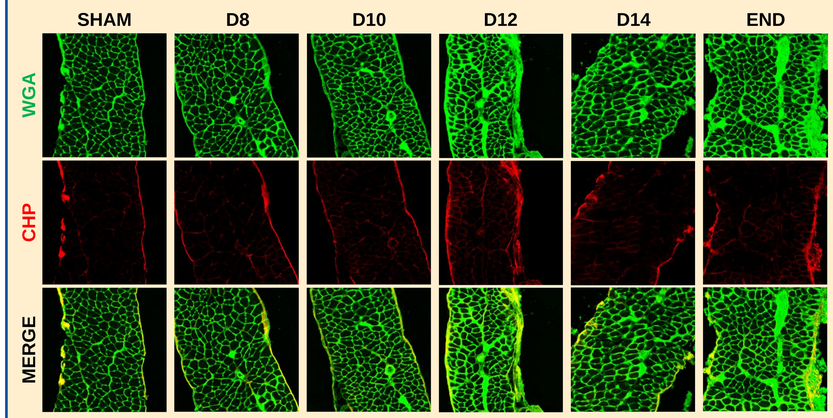

Fig.6 Extracellular matrix was identified via positive staining of wheat germ agglutinin (WGA, green). Newly damaged/remodeled collagen was identified via binding of collagen hybridizing peptide (CHP, red). D8-14 = 8-14 post-KPC cell inoculation. **P<0.01, ***P<0.001, ****P<0.0001.

IMMUNE CELL INFILTRATION

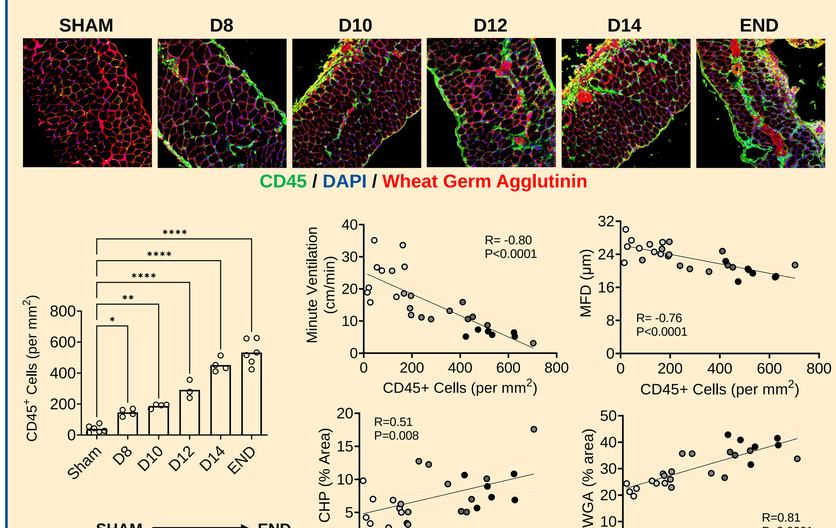

Fig.7 Immune cell (CD45+ leukocytes) infiltration progressively increased throughout tumor-burden. The degree of immune cell infiltration was negatively correlated to minute ventilation and DIA fiber size, and positively correlated to collagen remodeling (CHP) and the expansion of extracellular matrix (WGA). Time point indicated by point color. D8-14 = 8-14 post-KPC cell inoculation. *P<0.05, **P<0.01, ****P<0.0001.

CONCLUSIONS

- This is the first study to characterize the progression of respiratory muscle pathology and dysfunction in a pancreatic tumor model.
- These results highlight extracellular matrix remodeling and immune cell infiltration as key diaphragm pathologies associated with pancreatic cancer cachexia.
- Collectively, these findings demonstrate a rapid and robust model to investigate mechanisms involved at different stages of cachexia (i.e. pre-cachectic, cachectic, refractory/severe cachexia)

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