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Pancreatic Cancer-Induced Cachexia and Relevant Mouse Models

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Abstract

Pancreatic cancer is the 3rd leading cause of cancer death in the United States, with projections that it will become the second leading cause by the year 2030. It carries a dismal prognosis with a 5-year overall survival rate of less than 9% and is associated with numerous co-morbidities, the most notable being cachexia. Defined as the loss of muscle mass not reversible by conventional nutritional support, cachexia is seen in over 85% of pancreatic cancer patients and contributes significantly to mortality, where nearly 30% of pancreatic cancer deaths are due to cachexia rather than tumor burden. Therefore, there is an urgent need to identify the mechanisms behind the development of muscle wasting in pancreatic cancer patients and design novel therapeutics targeting cachexia. This review highlights the current understanding surrounding the mechanisms underpinning the development of cachexia in pancreatic cancer, as well as the current mouse models of pancreatic cancer-induced muscle wasting described in the literature.

Keywords

pancreatic cancer; cachexia; inflammation; pro-inflammatory cytokines

INTRODUCTION

Pancreatic cancer is currently the 3rd leading cause of cancer death, with projections that it will become the second leading cause of cancer death by the year 2030.¹ Due to a lack of early clinical signs, over 85% of patients present with advanced metastatic disease by the time of diagnosis.^{2,3} The prognosis for patients with metastatic disease remains poor, with a 5-year survival rate of only 2% and median survival time of 3–6 months.^{2,4} A major contributor to the low survival rate is the presence of cachexia, observed in over 85% of pancreatic cancer patients.⁵ Cancer cachexia is a complex syndrome with multifactorial

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causes, involving tumor- and host-derived signaling factors and alterations in metabolism that ultimately lead to muscle protein degradation.^{6,7} Importantly, the muscle wasting observed in cachectic patients is not reversible with conventional nutritional support.⁶ Although cancer patients often present with combined anorexia and cachexia, the loss of both skeletal muscle and fat, as well as the dysregulation of metabolic markers, separates cachexia from weight loss due to poor nutritional intake or malabsorption.⁷ The combination of cachexia, anorexia, insulin resistance, anemia, and chronic inflammation ultimately lead to a patient that is fatigued, immunosuppressed, unable to tolerate conventional chemotherapy, and at risk for death due to failure of respiratory muscles. In fact, nearly one-third of pancreatic cancer deaths are due to cachexia rather than tumor burden.^{8–10}

Advances in the understanding of cancer cachexia, in particular the pathogenesis of pancreatic cancer-induced cachexia, have identified prognostic and mechanistic information important in identifying potential therapeutic targets; however, current pharmacologic options for cachexia are limited.⁷ Therefore, further study is needed to improve clinical outcomes in pancreatic cancer, not only in addressing tumor burden but muscle wasting as well. This review is aimed at discussing the current understanding of the mechanisms behind pancreatic cancer cachexia, as well as the models presently available to study the efficacy of novel therapeutics.

MECHANISMS OF PANCREATIC CANCER CACHEXIA

Pro-inflammatory Cytokines and Activation of Ubiquitination

Pancreatic cancer, stromal, and immune cells within the tumor microenvironment will produce or otherwise induce the production of pro-inflammatory cytokines to promote growth, metastasis, and angiogenesis, and to evade apoptosis and cell-mediated immunity.^{6,11} In cachexia, these cytokines activate metabolic pathways that ultimately culminate in skeletal muscle wasting, such as increased proteolysis and decreased protein synthesis.¹² The most commonly implicated pro-inflammatory cytokines in cachexia include tumor necrosis factor (TNF)- α , interleukin (IL)-1, IL-6, and IL-8.⁶ IL-6 is overexpressed in pancreatic cancer and has been implicated in the development of cachexia and correlated with reduced survival.^{13–17} IL-6 mRNA expression was significantly increased in tumor samples and serum of patients with cachexia versus non-cachectic patients.¹³ In addition, the pro-cachectic factor TNF- α is also detectable in the serum of pancreatic cancer patients and has been associated with poor nutritional status in these patients.¹⁸ These cytokines activate signaling pathways that ultimately lead to skeletal muscle protein degradation; the two most described being the Jak2/STAT3 pathway and the NF- κ B pathway (Fig. 1).

IL-6 is produced from activated macrophages and/or other cells in the tumor microenvironment and stimulates the liver to induce an acute phase response.⁶ IL-6 binds to IL-6R α , which homodimerizes with gp130 to activate Jak2 (Janus kinase 2), which then recruits STAT3 (signal transducer and activator of transcription 3). STAT3 is phosphorylated on tyrosine 705, dimerizes, relocates to the nucleus, and binds to DNA as a transcription factor.¹⁹ Increased levels of phosphorylated STAT3 have been shown to be both necessary and sufficient for muscle wasting.^{6,20} Pharmacologic inhibition of STAT3 *in vitro* and *in vivo* was shown to reduce muscle atrophy downstream of IL-6 in experimentally induced

cancer cachexia.¹⁹ Activation of the Jak/STAT pathway has been correlated with a poor outcome in pancreatic cancer patients.¹⁴ However, little success has been observed with directly targeting STAT3 clinically in patients due to its poor solubility.

IL-6 and TNF- α both activate NF- κ B, a transcription factor known to inhibit skeletal muscle differentiation, and a positive feedback loop is generated, resulting in overstimulation of NF- κ B and degradation of muscle proteins through the production of catabolic cytokines.^{18,21,22} NF- κ B regulates two E3 ligases (atrogin-1 [muscle atrophy F box protein] and MuRF1 [muscle RING finger-containing protein 1]) of the ubiquitin-proteasome system, which plays a prominent role in skeletal muscle degradation in cancer cachexia.²³ These ligases mediate sarcomeric breakdown and inhibition of protein synthesis. In particular, MuRF1 is responsible for mediating the ubiquitination of the thick filament of the sarcomere.⁶ Active NF- κ B may also inhibit muscle repair due to upregulation of transcription factors that can suppress the transcription of MyoD, a protein involved in satellite cell proliferation following muscle injury.²¹ Inhibition of NF- κ B through proteasome inhibitors, or decreased production of IL-6 and TNF- α with low dose anti-inflammatory agents, has been shown to prevent body weight loss and muscle atrophy in mouse models.^{18,22}

In addition, increased expression of genes encoding other pro-inflammatory markers, including acute-phase response proteins fibrinogen- α , α_1 -antitrypsin, and α_2 -macroglobulin, have been identified in the skeletal muscle of cachectic pancreatic cancer patients. Furthermore, a recent study identified high levels of midkine,²⁴ CXCL-16, IL-6, TNF- α , and low IL-1 β to be correlated with the development of weight loss in newly diagnosed pancreatic cancer patients.²⁵ Taken together, these results suggest that the pro-inflammatory response likely plays a major role in the pathogenesis of pancreatic cancer cachexia.

Increased Proteolysis

Myostatin and activin are TGF- β superfamily ligands involved in skeletal muscle degradation.²⁶ Myostatin, which is secreted from muscle cells, is a key negative regulator of muscle growth, as a deficiency in myostatin results in muscle hypertrophy.²⁷ Activin A is formed from two inhibin beta chains and is involved in cell growth and differentiation.²⁸ Both ligands bind to its respective type II activin receptor on the muscle cell membrane, leading to recruitment and activation of type I receptors, which then phosphorylate SMAD2 and SMAD3. These factors, along with SMAD4, translocate to the nucleus and regulate transcriptional responses leading to muscle wasting.²⁹ Studies have shown that serum levels of activin and myostatin are increased in patients with cachexia associated with colorectal and lung cancer³⁰. A recent study by Zhou et al. showed that antagonism of the type II activin receptor can inhibit myostatin and activin-mediated Smad2/3 signaling and prevent muscle wasting in mouse models of cancer cachexia.³¹ While work still needs to be done to evaluate the role of activin and myostatin in pancreatic cancer cachexia, preliminary evidence suggests that pancreatic cancer patients with high levels of circulating activin A have a shorter progression free survival and that mice inoculated with MIA PaCa-2 pancreatic cancer cells overexpressing the inhibin beta A subunit (*INHBA*) have decreased body weight and atrophied cardiac muscle compared to mice injected with tumor cells only.

³² Additionally, TGF- β blockade using a monoclonal antibody was able to improve body weight, lean body mass, and bone mineral density in a syngeneic mouse model of pancreatic cancer-induced cachexia.²⁴

Decreased Protein Synthesis

The IGF1/PI3K/Akt signaling pathway is major anabolic pathway involved in muscle development and regeneration.³³ IGF1 (insulin-like growth factor 1) binds to its receptor and activates PI3K, which then activates Akt signaling. Akt suppresses protein breakdown and promotes muscle growth by blocking the repression of mTOR, which phosphorylates targets that promote myogenesis. Akt also inactivates forkhead box O proteins (FoxO1, FoxO3, FoxO4), which leads to protein ubiquitination and degradation of muscle cells through autophagy^{26,34} and increased expression of ubiquitin ligases atrogen-1 and MuRF1.³³ In a study evaluating muscle from cachectic pancreatic cancer patients, the Akt protein level was decreased by 55% and levels of phosphorylated mTOR were decreased by 82% compared to patients without cachexia. Additionally, phosphorylation of FoxO1 and FoxO3a was reduced by 4-fold. The authors concluded that cachexia in pancreatic cancer patients was associated with loss of Akt-dependent signaling in human skeletal muscle with decreased activity of regulators of protein synthesis and disinhibition of protein degradation.³³ Other studies have identified AKT1 polymorphisms in pancreatic cancer patients with cachexia.³⁵ Coming full circle, myostatin and activin can suppress Akt, thus disinhibiting FoxO3, and can upregulate expression of the E3 ligases MuRF1 and atrogen 1 of the ubiquitin-proteasome pathway.^{26,36}

Altered Lipid Metabolism

Felix et al. used a SELDI (Surface Enhanced Laser Desorption) ProteinChip system to analyze the serum from patients with pancreatic cancer to identify a subgroup of cachectic patients.¹⁰ They found that glucagon-like peptide-1 (GLP-1), apolipoprotein C-II, and apolipoprotein C-III were elevated in cachectic patients. GLP-1 acts on the hypothalamus to inhibit food intake and stimulates satiety.³⁷ Both lipoproteins are involved in lipid metabolism and are associated with decreased lipogenesis and negative energy balance.³⁸ The study also identified an increase in zinc- α 2-glycoprotein (ZAG), a protein that stimulates lipolysis in adipocytes and causes weight loss without a reduction in food intake.³⁹ Therefore, it is likely that dysregulation of lipid metabolism, in addition to altered protein metabolism, also plays a role in the pathogenesis of pancreatic cancer cachexia.

Anorexia and Secondary Effects

In addition to the dysregulation associated with pro-inflammatory cytokines and unequal balance between protein synthesis and protein degradation, patients with pancreatic cancer have numerous other factors contributing to the development of body weight loss. Decreased nutritional intake can be associated with stenosis of the duodenum, and maldigestion can be caused by exocrine pancreatic insufficiency, gastroparesis, or delayed gastric emptying. Bloating, taste alterations, and nausea are also associated with decreased nutrition in these patients.^{7,40} Furthermore, cytokines implicated in the development of cachexia, including TNF- α , IL-1, and IL-6, are also thought to contribute to the development of anorexia through modulation of signaling pathways that mediate the balance between stimulating food intake and inhibiting food intake. Although signals indicating an energy deficit are sent

to the hypothalamus, these cytokines prevent the hypothalamus from inducing an appropriate response to increase food intake.⁴¹ Therefore, pancreatic cancer patients often experience decreased food intake and a negative energy balance in addition to cachexia, further driving the loss of body mass.

MOUSE MODELS OF PANCREATIC CANCER-INDUCED MUSCLE WASTING

Although research specifically focused on cancer cachexia has intensified over the last several years, evaluation of the mechanisms contributing to the development of muscle wasting using human tissue has been scarce. Therefore, the majority of studies have focused on using animal models to elucidate mechanisms and evaluate novel therapeutics.⁴² The most commonly used animal models include the colon-26 carcinoma model and Lewis lung carcinoma model.^{6,43} Although these models have contributed significantly to our understanding of cancer cachexia, they may not be representative of the complex interactions contributing to the development of cachexia in pancreatic cancer patients. More recent literature describes the characterization of specific pancreatic cancer cachexia models or the development of a mouse model to then evaluate potential therapeutics (Table 1).

Orthotopic Models

The most common models developed to evaluate pancreatic cancer-induced muscle wasting have involved the use of orthotopic implantation of human or mouse pancreatic cancer cell lines into the pancreas of mice.

PANC-1 Orthotopic Model

In this model, 1×10^6 PANC-1 cancer cells were injected subcutaneously into the flank or orthotopically into the pancreas of 8-week old female NOD-SCID IL2 receptor gamma chain knockout (NSG) mice.⁴⁴ Mice were euthanized after 10 weeks. Flank tumors did not produce a significant change in body weight or tibialis anterior (TA) muscle weight compared to tumor free mice. However, the tumor-free body weight of orthotopic tumor-bearing mice was 15% less than controls and TA muscle weighed 21% less. Cross-sectional area (CSA) of muscle from these mice was 50% decreased compared to sham mice. These changes in muscle were associated with upregulation of cachexia biomarkers in the PANC-1 orthotopic mice, including Forkhead Box O (FoxO)-1 and FoxO3, the E3 ligases atrogin-1 and MuRF1, Stat3, and Socs3. Furthermore, PANC-1 tumor-bearing mice had higher splenic concentrations of the pro-inflammatory cytokines IL-6 and IL-8. Chemokines, including IP10, MCP1, MIP2, RANTES, and MIP1 β were also significantly increased in this model. The authors concluded that the PANC-1 tumor-bearing model demonstrates a chemokine-centric mechanism to induce muscle wasting, and that orthotopic injection of PANC-1 cells was necessary to induce muscle wasting using this cell line.

L3.6pl Orthotopic Model

In addition to evaluating PANC-1, Delitto et al. also evaluated a subcutaneous flank and orthotopic model using the L3.6pl pancreatic cancer cell line.⁴⁴ This cell line is a metastatic variant derived from the liver following repeated orthotopic injections of the pancreatic cancer cell line COLO-357 into athymic nude mice.⁴⁵ These mice were sacrificed 4–6

weeks following injection. Unlike the PANC-1 model, there were no differences in body weight in tumor free mice versus flank or orthotopically injected mice. However, both models demonstrated 14–15% decrease in TA muscle weights (flank and orthotopic, respectively). Fiber CSA was decreased 27% in flank mice, and 40% in orthotopic mice. Similar to the PANC-1 orthotopic model, the cachexia biomarkers FoxO1, FoxO3, atrogin-1, MuRF-1, Stat3, and Socs3 were significantly increased in both models, albeit more robust in the orthotopic model. Finally, pro-inflammatory cytokines, including IL-8, TNF- α , and IL-1 β were increased in the spleens of both models, whereas only IL-6 was increased in orthotopic tumor bearing mice. Anti-inflammatory markers IL-4 and IL-10 were also significantly decreased in orthotopic mice. Taken together, the authors concluded that both flank and orthotopic L3.6pl tumors produce a pro-inflammatory systemic environment contributing to muscle wasting with a more substantial effect in orthotopic mice.

S2-013 Orthotopic Models

S2-013 is a subline of the human pancreatic cancer cell line SUIT-2 derived from a liver metastasis. In a 2014 study, Shukla et al. devised a cachexia model through orthotopic injection of 5×10^5 S2-013 pancreatic cancer cells into the pancreas of athymic nude mice in order to evaluate the anti-cachectic efficacy of a ketogenic diet.⁴⁶ S2-013 tumor-bearing mice fed a ketogenic diet demonstrated a 20% increase in carcass weight and a 45% increase in muscle weight compared to control fed S2-013 tumor-bearing mice. While cell culture experiments using C2C12 myotubes demonstrated that ketone bodies can inhibit myotube degradation as well as reduce expression of the pro-cachexia markers atrogin-1 and MuRF1, these markers were not assessed in muscle from S2-013 tumor bearing mice, nor were sham injected mice used as a control to evaluate the pro-cachexia phenotype of S2-013 cells. Furthermore, the ketogenic diet significantly decreased tumor burden in tumor-bearing mice, making it difficult to elucidate the anti-cachectic effects of ketone bodies on muscle versus a secondary effect of decreased tumor mass.

In 2015, Shukla et al. published another study using the S2-013 pancreatic cancer cell line as an orthotopic model to evaluate the anti-cachectic efficacy of silibinin, a bioactive component of Milk thistle (*Silybum marianum*) seeds.⁴⁷ Again, 5×10^5 S2-013 pancreatic cancer cells were injected into the pancreas of 6–8 week old athymic nude mice and mice were sacrificed 4 weeks after injection and 3 weeks after treatment with silibinin. In this study, non-tumor bearing mice were used as controls. S2-013 tumor bearing mice demonstrated a significant decrease in carcass weight, gastrocnemius muscle weight, grip strength (a measure of muscle function), and decreased cross-sectional area versus tumor-free control mice. Treatment with silibinin significantly improved these parameters compared to vehicle treated tumor-bearing mice, albeit not to the level of tumor free mice. Furthermore, mRNA levels of Atrogin-1 and MuRF1 were 6- to 8-fold higher in S2-013 tumor bearing mice versus tumor-free mice, with a significant decrease in the expression of these markers in silibinin treated mice. Finally, the pro-inflammatory cytokines TNF- α and IL-6 were increased in tumor-bearing mice. Although food consumption in silibinin treated mice was significantly greater than control mice, no comparison to tumor-bearing mice was made; therefore, the contribution of anorexia to the development of body weight loss in this model is unknown. However, the S2-013 orthotopic model appears to recapitulate a pro-

cachexia phenotype similar to the orthotopic PANC-1 and L3.6pl models and was a suitable model to evaluate the anti-cachectic efficacy of silibinin.

COLO-357 Tumor Fragment Orthotopic Model

COLO-357 human pancreatic cancer cells were injected subcutaneously into athymic nude mice.⁴⁸ Once tumors became established, 1mm³ tumor fragments were surgically implanted into the pancreas of 6- to 8-week old athymic nude mice. At 60 days post-implantation, mice were euthanized when they began to show signs of significant morbidity, including ascites, jaundice, and widespread metastasis. Tumor size and muscle weights (gastrocnemius and soleus muscles) were recorded. Food consumption was reported to be similar between tumor-bearing mice and tumor-free mice. The gastrocnemius and soleus muscle of tumor bearing mice was approximately 60% and 50%, respectively, decreased compared to the muscle weights of control mice. Additionally, the primary tumor weight was recorded to be approximately 5 grams in this model. Although final body weights were not reported, the protocol describes use of nude mice weighing approximately 20 to 25g at the start of the study. Therefore, it is likely that the final tumor burden was approximately 20% of the animal's body weight or greater. However, the model was successfully used to demonstrate the anti-cachectic effects of bortezomib, a proteasome inhibitor, in improving muscle weight and decreasing tumor burden.

MIA PaCa-2/*INHBA* Orthotopic Model

INHBA encodes the beta A subunit of the activin protein. Based on previous studies demonstrating the pro-cachectic activity of activating,^{49–51} Togashi et al. evaluated the pro-cachectic ability of *INHBA* over-expression in the MIA PaCa-2 human pancreatic cancer cell line.³² While cachexia was not the primary focus of their study, they did show that athymic nude mice injected subcutaneously into the flank with 5×10^6 *INHBA* transfected MIA PaCa-2 cells produced a larger tumor volume, shorter survival period, and decreased body weight at 22 and 26 days post-innoculation compared to control MIA PaCa-2 tumor bearing mice. Furthermore, heart muscle fibers were atrophied compared to control mice. Although a full assessment of the pro-cachectic phenotype of MIA PaCa-2/*INHBA* tumor-bearing mice was not made, it provides evidence that activin may play a role in pancreatic cancer-induced cachexia.

Syngeneic Pan02 Intraperitoneal (i.p.) Model

In this study, Greco et al. created a syngeneic pancreatic cancer cachexia model by injecting 10×10^6 mouse-derived pancreatic cancer cell line Pan02 intraperitoneally into male C57BL/6 mice.²⁴ These mice developed peritoneal carcinomatosis and liver metastasis 3 weeks post-injection and the majority of mice succumbed to disease burden within 45 days. Body weight loss began around 2 weeks post-injection. Dual-energy x-ray absorptiometry (DEXA) showed that Pan02 tumor-bearing mice lost significantly greater amounts of fat mass and muscle mass, as well as had decreased bone mineral content and bone mineral density. Upper arm circumference was also decreased in Pan02 mice. Similar to other models, levels of the E3 ligases atrogin-1 and MuRF1 were increased in the muscle of Pan02 mice, both in gene expression and protein expression. Furthermore, zinc alpha glycoprotein (ZAG), a marker of lipolysis, was increased. Again, levels of pro-inflammatory cytokines

MCP-1 and IL-6 were elevated in tumor-bearing mice. This model was also successfully used to evaluate potential therapeutic options, in this case toll like receptor 9 (TLR9) inhibition and TGF- β inhibition. While inhibition using a TLR9 inhibitor was not effective in ameliorating cachexia in this mouse model, the group did find that targeting TGF- β using a neutralizing monoclonal antibody was effective in decreasing weight loss, fat loss, and loss of bone density compared to untreated tumor-bearing mice.

Chemically-induced Pancreatic Cancer Cachexia Models

Gemcitabine-induced Cachexia—In this model, 4- to 6- week old athymic nude mice were implanted with 1mm³ tumor fragments derived from tumors grown following subcutaneous injection of 3×10^6 SW1990 human pancreatic cancer cells in the flank of nude mice.⁵² Chemotherapy-induced cachexia was established by intraperitoneal injection of 50 mg/kg gemcitabine on days 10, 13, and 16 after tumor implantation and mice were sacrificed at day 28 post-implantation. Gemcitabine caused an approximately 7% decrease in body weight compared to control mice. These mice also had a mild decrease in food intake as well as subcutaneous fat and arm circumference. This study established the ability of gemcitabine, considered a standard of care chemotherapeutic in pancreatic cancer, to induce mild cachexia. Treatment with Mirtazapine, an antidepressant that can stimulate appetite, was able to improve food intake and body weight in this mouse model, but could not slow the loss of subcutaneous fat and skeletal muscle.

Genetically Engineered Mouse Models of Pancreatic Cancer

KPC/INK4 (*Pdx1-cre;LSL-Kras^{G12D};INK4a/ar^{fl/fl}*) Transgenic Mouse Model—Transgenic mouse models are frequently used to study pancreatic cancer due to their ability to recapitulate the many aspects of human pancreatic cancer, including histopathologic progression from pre-neoplastic Pan-IN lesions to invasive pancreatic ductal adenocarcinoma (PDAC), genomic instability, metastasis, and cachexia.⁵³ The KPC/INK4 model produces spontaneous PDAC and death at 8–12 weeks of age.⁵⁴ Cachectic mice were identified by their reduced body weight, muscle atrophy, decreased activity, and presence of pancreatic tumors. Through microarray profiling of cachectic muscle from KPC/INK4 transgenic mice, Gilabert et al. were able to show upregulation of several pro-cachexia pathways, including Jak2/STAT3, NF- κ B, IL-1, IL-6, and lipolysis inducing factor⁵⁵ compared to muscle from control mice (*LSL-Kras^{G12D};INK4a/ar^{fl/fl}*, which have the same genetic background but do not develop tumors or cachexia). Treatment with a Jak2 inhibitor was able to improve body weight to levels similar to control mice without a significant effect on tumor weight compared to vehicle treated tumor bearing mice. Furthermore, levels of phosphorylated Jak2 in liver, white adipose tissue, and skeletal muscle were decreased in treated mice. Levels of IL-6 in treated mice were also significantly lower than vehicle tumor-bearing mice, albeit over twice the levels seen in tumor-free mice.

KP^{fl/fl}C (*LSL-Kras^{G12D};Trp53^{flx/flx};Pdx-1-Cre*) Transgenic Mouse Model—Work in the laboratory of the first author evaluating the anti-tumor and anti-cachectic activity of AR-42 in mouse models of pancreatic cancer utilized the KP^{fl/fl}C mouse model, which recapitulates the functional heterogeneity of pancreatic cancer.⁵⁶ We had previously shown that AR-42 was able to ameliorate muscle wasting in mouse models of cancer cachexia⁵⁷.

Here, we were able to show that the $KP^{fl/C}$ mouse model produces decreased cross-sectional area of muscle fibers as well as decreased grip strength.⁵⁸ AR-42 was able to preserve muscle fiber size and improve grip strength from 4 to 8 weeks of age in treated mice versus vehicle treated mice. Furthermore, treatment with AR-42 resulted in decreased expression of the cachexia biomarker MuRF1 due to HDAC inhibition. Unfortunately, age-matched tumor free mice were not available for comparison of muscle indices.

SUMMARY AND PERSPECTIVES

Clearly, over the last 5 years, there have been many advances in the study of pancreatic cancer-induced cachexia as well as efforts to recapitulate it through mouse models. Further understanding in the roles of pro-inflammatory cytokines and cellular and metabolic signaling pathways has provided the field with potential targets in which to develop novel therapeutic agents. Additionally, well-characterized mouse models are becoming increasingly available in which to study the efficacy of these therapeutics. However, much work still needs to be done to further elucidate the mechanisms behind pancreatic-cancer induced cachexia in order to develop therapeutics with meaningful clinical outcomes.

Orthotopic pancreatic cachexia models have become the most common established models in the literature. The advantages of these models is the use of human pancreatic cancer cell lines or patient derived tissue with numerous and varying genetic abnormalities and the ability to modulate the expression of various targets of interest, the relative in-expense in creating these models versus maintaining a breeding colony of GEMMs, the relatively long time frame from injection until death, and ability to age and weight match control versus tumor-bearing mice.^{44,59,60} However, there are inherent disadvantages to these models, in particular the necessity of using immunocompromised mice, as well as the technical expertise to perform the surgeries, variable metastatic capabilities of different cell lines, and the significant tumor burden relative to the animal's body weight.^{24,48,61} Syngeneic models and genetically engineered models provide the immunological and microenvironment parameters; however, it is unknown to what extent mouse cachexia differs from the human syndrome, and these models often produce rapidly growing and lethal tumors that make it impossible to evaluate treatment effects.^{54,62}

While there are several pros and cons to the use of orthotopic versus chemically induced versus transgenic mouse models, one of the biggest challenges in evaluating and comparing the models is the inconsistency in measured cachexia parameters. As discussed earlier, cachexia is not merely a decrease in body weight, but is in fact a syndrome also characterized by decreased food intake, loss of muscle and adipose mass, decreased muscle function, and modulation of numerous cytokines, transcription factors, and metabolic parameters within the muscle and systemically.⁷ Additionally, human clinical trials testing novel anti-cachectic therapies often fail due to an overall lack of consistent diagnostic criteria and measurable parameters.^{42,63} As more knowledge becomes available concerning the multiple facets of the cachexia syndrome in human patients and what parameters equate to therapeutic success, characterization of novel cachexia models should include the analysis of as many of these parameters as possible.

Perhaps the largest hurdle in all of the models is how accurately they recapitulate the human syndrome. Tumors in these mouse models often approach 10–20% of the body weight of the mouse, which is not the case in the development of cachexia in people. Pancreatic cancer tissue can also be comprised of almost 50% stroma and we know from our work and others that the stromal compartment secretes a host of pro-inflammatory cytokines¹¹ that can potentially be a leading driver of cachexia. It will be essential that animal models consider the importance the tumor microenvironment may have in driving pancreatic cancer-induced cachexia. Future experimental design incorporating stromal cells into pancreatic cachexia models will be important for replicating the tissue microenvironment and the pro-inflammatory factors that exist in patients. Additionally, there is evidence that many of these factors (IL-6, TNF- α , IL-1) which promote pancreatic cancer-induced cachexia in the above murine models are elevated and drivers of this syndrome in patients.^{64,65} In many other cancer types (breast, ovarian, and esophageal), cachexia is characterized by anorexia, malnutrition, and treatment induced effects which is often not directly induced by cytokine or factors secreted by the tumor and becomes apparent only in the late stages of disease progression.^{66–69} However, in pancreatic cancer, the syndrome of cachexia is present in over 70% of newly diagnosed pancreatic cancer patients.⁷⁰ This highlights the importance of designing murine models that replicate the active wasting of the muscle driven by factors secreted by the pancreatic tumor microenvironment early in the disease process.

Ultimately, cachexia is a heterogeneous syndrome that is influenced by numerous different variables, including nutritional status, age and gender differences, prior therapies, and other co-morbidities.⁴² Nonetheless, similarities between animal models and existing data on pancreatic cancer-induced muscle wasting, such as elevations in pro-inflammatory cytokines, activation of pro-cachectic signaling pathways, including Jak2/STAT3 and NF- κ B, and perturbations in metabolism provide a solid starting point in which to develop targeted therapeutics. As more models are developed, researchers will have the opportunity to test therapeutics in a variety of different models, further supporting efficacy and mechanism of action for translation into human clinical trials. A major limitation of many of the current animal models of pancreatic cancer-induced cachexia is that they utilize human cell lines in immunocompromised mice which lack an intact immune system. These models do not incorporate the complex tumor microenvironment composed of stroma, pancreatic stellate cells, and immune cells. Many of the pro-inflammatory cytokines and factors that drive pancreatic cancer-induced cachexia can also modulate different immune cell responses and cellular populations. These immune cells may not only be responsible for secreting inflammatory cytokines but could also infiltrate adipose and muscle tissue leading to inflammation, further driving the cachexia syndrome.^{71–73} Future models using immunocompetent mice with either spontaneous genetically engineered pancreatic tumors or syngeneic tumor implantation could address these issues. Importantly, these models should aim to produce slow growing tumors in order to investigate the mechanisms driving cachexia early in the disease process, as this is a unique feature in pancreatic cancer compared to cachexia development in other cancers which occurs as a component of late stage disease and therapeutic intervention. Additionally, genetic models deleting pro-inflammatory receptors specifically on muscle cells in murine models could help uncover the mechanisms driving cachexia *in vivo*. Designing pancreatic cancer animal models that will be able to

assess cachexia in an immunocompetent systems using relevant mouse models will be important for accurately recapitulating and understanding this disease as well as promoting the successful development of novel therapeutic approaches.

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FIGURE 1.

Pro-inflammatory cytokines are drivers of cancer-induced cachexia. Pro-inflammatory cytokines IL-6 and TNF- α activate downstream signaling of NF- κ B resulting in overstimulation of NF- κ B and degradation of muscle proteins through the production of catabolic cytokines. Cytokines can also activate Jak2/STAT3 signaling pathways that can lead to skeletal muscle protein degradation.

TABLE 1

Summary of Published Pancreatic Cancer Cachexia Models

Models	Method	Mouse Strain	Timeline	Parameters Evaluated
Orthotopic Mouse Models				
<i>PANC-1</i> Delitto et al, 2017 ⁴⁴	1×10 ⁶ tumor cells injected into pancreas	8-week old female NSG [*] mice	10 weeks	Body weight Muscle weight Muscle cross sectional area Cachexia biomarkers in muscle (mRNA) Cytokine/chemokine (splenic origin)
<i>L3.6pl</i> Delitto et al, 2017 ⁴⁴	1×10 ⁶ tumor cells injected into pancreas	8-week old female NSG mice	4–6 weeks	Body weight Muscle weight Muscle cross sectional area Cachexia biomarkers in muscle (mRNA) Cytokine/chemokine (splenic origin)
<i>S2-013</i> Shukla et al, 2014 ⁴⁶	5×10 ⁵ tumor cells injected into pancreas	6- to 8-week old athymic nude mice	4 weeks	Body weight Muscle weight Grip strength Muscle cross-sectional area Cachexia biomarkers in muscle (mRNA) Cytokine/chemokine (serum)
<i>COLO-357</i> Jones-Bolin et al, 2007 ⁴⁸	1 mm ³ tumor fragments sutured to pancreas	6- to 8-week old athymic nude mice	60 days	Tumor size Muscle weight Food consumption
<i>MIA PaCa-2/INHBA over-expressing</i> Togashi et al, 2015 ³²	5 × 10 ⁶ <i>INHBA</i> transfected MIA PaCa-2 cells	Athymic nude mice	4 weeks	Tumor size Survival Body weight Cardiac muscle fiber diameter
<i>Syngeneic Pan02</i> Greco et al, 2015 ²⁴	10×10 ⁶ tumor cells intraperitoneal (i.p.)	Male C57BL/6 mice	45 days	Fat mass Muscle mass Bone mineral content/density Upper arm circumference Cachexia biomarkers in muscle (mRNA and protein) Cytokines (serum)
Chemically-induced models				
<i>Gemcitabine-induced SW1990</i> Jiang et al, 2012 ⁵²	1 mm ³ tumor fragments into right dorsal flank	4–6 week old athymic nude mice i.p. injection of 50mg/kg gemcitabine days 10, 13, 16 post-implantation	4 weeks	Body weight Food intake Subcutaneous fat circumference Upper arm circumference
Genetically engineered models				
<i>KPC/INK4</i> Gilabert et al, 2014 ⁵⁴	Transgenic model	<i>Pdx1-cre;LSL-Kras^{G12D};INK4a/ar^{fl/f}</i>	8–12 weeks	Genome-wide expression profiles of white adipose tissue, skeletal muscle, and liver Body weight Pancreas weight Western blot analysis of phosphorylated Jak2 in liver, white adipose, and skeletal muscle Serum IL-6 levels
<i>KP^{fl/fl}C</i> Henderson et al, 2016 ⁵⁸	Transgenic model	<i>LSL-Kras^{G12D};Trp53^{fllox/flox};Pdx-1-Cre</i>	8 weeks	Muscle cross sectional area Grip strength Cachexia biomarkers in muscle (protein)

* NSG: non-obese diabetic, severe combined immunodeficient (NOD-SCID) IL-2 receptor gamma chain knockout mice