Cardiac alterations in cancer-induced cachexia in mice

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Abstract. Cachexia is a common syndrome in advanced cancer patients and causes up to 22% of cancer-related deaths. It remains elusive whether cancer cachexia causes heart failure. We investigated the effect of cancer cachexia on heart function and cardiac muscle structure in a mouse model. Male CD2F1 mice were inoculated with either colon-26 adenocarcinoma cells (Tumor group) or vehicle (PBS) (No Tumor group and Pair-fed group). Heart function as measured by fractional shortening in vivo using transthoracic echocardiography was performed on day 14 after tumor or PBS inoculation. At necropsy (day 17), hearts were collected for histology, transmission electron microscopy, RT-PCR and SDS-PAGE analysis. Mice from the Tumor group displayed a significantly reduced fractional shortening compared to mice in the No Tumor and Pair-fed groups. In hearts of the Tumor mice compared to the other groups, there was marked fibrosis and transmission electron microscopy revealed disrupted myocardial ultrastructure. Gene expression of troponin I, a regulator of cardiac muscle contraction, was reduced. Moreover, both mRNA and protein levels of myosin heavy chain (MHC) were altered whereby MHCα (adult isoform) was decreased and MHCβ (fetal isoform) was increased indicating reactivation of the fetal gene expression pattern. In conclusion, heart function was diminished in mice with tumor-induced cachexia, and this impaired function was associated with increased fibrosis, disrupted myocardial structure and altered composition of contractile proteins of cardiac muscle.

Introduction

Cachexia is a common complication of cancer, refers to the involuntary loss of more than 5% of body weight within 6 months (1) and may be responsible for as many as 22% of cancer deaths (2,3). Cancer cachexia is associated with weakness and fatigue, and it significantly impairs quality of life, response to anticancer therapy and survival (2,4). Cancer cachexia is a syndrome with multiple etiologies including anorexia, systemic inflammation and metabolic dysregulation. The net result is both depletion of adipose tissue depots and skeletal muscle atrophy (5).

While considerable research has addressed the mechanisms of skeletal muscle atrophy in cancer cachexia, the alteration of cardiac muscle structure and metabolism is not well understood. In addition, it remains elusive whether cancer cachexia causes heart failure. A recent study reported that cancer cachexia caused apoptosis-mediated multiple organ failure in rabbits, but the heart was spared from this effect (6). On the contrary, several studies have reported altered metabolism (7) as well as increased protein loss in hearts of patients and mice with cancer cachexia (8-11). Despite a loss of cardiac contractile and myofibrillar proteins, heart performance did not seem to be impaired (12,13). This was attributed to an adaptive response to protect heart function (14,15). An alternative explanation for the loss of cardiac contractile mass is anorexia, which lead to increased degradation and decreased synthesis of protein (16). A limitation to these studies is that heart function was evaluated ex vivo (7,13,14). The purpose of this study was to evaluate heart function in a mouse model of cancer cachexia in vivo, investigate whether functional changes are dependent on reduced food intake and explore whether the changes in function are associated with cellular abnormalities.

Materials and methods

Experimental animals and design. Five-week-old, male CD2F1 mice (BALB/c x DBA/2; Charles River Laboratories, Wilmington, MA) were housed five per cage at 22±0.5˚C on a 12-h light/dark cycle. The mice were fed AIN-93G purified pellet diet (Research Diets, New Brunswick, NJ) containing 7% fat by weight. When mice weighed approximately 20 g, they were randomly assigned to one of three groups: No Tumor (n=10), Tumor (n=15), or Pair-fed (n=10). On study day 0, the mice were inoculated subcutaneously in the right flank with either 1x10⁶ colon-26 adenocarcinoma cells suspended in 100 μl PBS (Tumor group) or vehicle (PBS; No Tumor and Pair-fed groups). Mice in the Pair-fed group were fed the same amount of food consumed by Tumor group. When there
Target gene expression was normalized to the endogenous from Applied Biosystems (TaqMan Gene Expression Assays). RT-PCR analysis was performed with pre-designed primers.

**Histological analysis.** Hearts were rapidly excised and a histological analysis was performed using a transmission electron microscope at 80 kV. Hearts were sliced into 1-mm3 cubed pieces and immediately put into 2% aqueous uranyl acetate followed by Reynolds lead resin and polymerizing at 60˚C for 16-24 h. Sections were cut at 80 nm on a Reichert Ultracut E ultramicrotome, stained with 5 μm were stained with Masson’s trichrome, and then analyzed by light microscopy. Fibrosis was independently analyzed in a blinded manner by three evaluators. For each sample, 4-6 microscopic fields were chosen and scored numerically for the extent of fibrosis relative to a positive control.

**Transmission electron microscopy.** Ventricles from hearts were homogenized and loaded onto a 7% SDS-PAGE gel (20). Gels were run at 230 V for 21 h at 8˚C, and then silver stained according to methods of Giulian et al (20).

**Histological analysis.** Hearts were rapidly excised and a portion of the ventricles was fixed in 10% neutral-buffered formalin, then paraffin embedded. Sections with a thickness of 5 μm were stained with Masson’s trichrome, and then analyzed by light microscopy. Fibrosis was independently analyzed in a blinded manner by three evaluators. For each sample, 4-6 microscopic fields were chosen and scored numerically for the extent of fibrosis relative to a positive control.

**RT-PCR analysis.** RNA was isolated from the ventricles with TRIzol reagent (Invitrogen, Carlsbad, CA) and reverse transcribed with the High Capacity cDNA Archive kit (ABI, Foster City, CA) according to the manufacturer’s protocols. RT-PCR analysis was performed with pre-designed primers from Applied Biosystems (TaqMan Gene Expression Assays). Target gene expression was normalized to the endogenous control 18S rRNA amplified in the same reaction and expressed as 2-ΔΔCT relative to the No Tumor group (19).

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**Statistical analysis.** Data were analyzed using MINITAB 15 (State College, PA) and are presented as mean ± standard error of mean (SEM). Differences among Tumor, No Tumor and Pair-fed groups were detected by one-way ANOVA and Tukey’s post-hoc analyses. In Figs. 5 and 6, differences between the Tumor and No Tumor groups were analyzed by unpaired, two-sample t-tests. Differences were considered significant at P<0.05.

**Results**

Colon-26 adenocarcinoma induces cachexia. The starting body weights among the three groups were not different. There was a 23% difference in body weight between the Tumor and No Tumor groups at the end of the study (Fig. 1). The Tumor group showed continual growth throughout the study, as evidenced by continuous body weight increases. The body weight in the Tumor group was significantly lower than the No Tumor group beginning at 12 days after tumor inoculation and this difference continued throughout the rest of the study (Fig. 1). Food intake in Tumor mice was 72% of that in No Tumor mice (data not shown). Mice in the Pair-fed group demonstrated a growth curve with similar pattern to Tumor mice, despite slightly smaller extent of body weight loss (Fig. 1).

The absolute masses of skeletal muscle and epididymal adipose were significantly less in the Tumor group, compared to the No Tumor group (Table I). In contrast, although the mice of the Pair-fed group lost a similar amount of body weight, the mass of skeletal muscle was not significantly different from the No Tumor group (Table I). In contrast, although the mice of the Pair-fed group lost a similar amount of body weight, the mass of skeletal muscle was not significantly different from the No Tumor group (Table I).
weight as the tumor-bearing mice, their muscle weights were markedly higher. When normalized to body weight, the percentage of muscle weight in the Pair-fed mice was higher than that of the No Tumor mice (Table I). These results support the idea that decreased food consumption alone is not sufficient to cause skeletal muscle wasting in this model. Absolute weight of the heart was lower in tumor-bearing mice compared to No Tumor mice, however when normalized to percentage of tumor-free body weight, heart weight was higher in Tumor mice. Both liver and spleen weight were significantly higher in tumor-bearing mice compared to those in No Tumor groups. Echocardiographic assessment revealed that tumor-bearing mice displayed a significantly reduced heart rate as well as fractional shortening compared to those in No Tumor and Pair-fed groups (Table II, Fig. 2).

Table I. Tissue and organ weights.

<table>
<thead>
<tr>
<th>Absolute weight (mg)</th>
<th>Percentage of body weight (%)</th>
<th>Percentage of tumor-free body weight (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Tumor</td>
<td>Tumor</td>
</tr>
<tr>
<td>Skeletal musclesd</td>
<td>714±17.9</td>
<td>467±12.7</td>
</tr>
<tr>
<td>Epidydimal adipose</td>
<td>772±46.0</td>
<td>51.1±10.8</td>
</tr>
<tr>
<td>Heart</td>
<td>103.7±3.2</td>
<td>81.7±2.4</td>
</tr>
<tr>
<td>Liver</td>
<td>983±38.8</td>
<td>903.6±39.2</td>
</tr>
<tr>
<td>Spleen</td>
<td>63.3±3.3</td>
<td>151.9±10.4</td>
</tr>
</tbody>
</table>

Weights of tissues and organs are expressed as absolute weight, percentage of body weight and percentage of tumor-free body weight in No Tumor (n=10) group, Tumor (n=14) group and Pair-fed (n=10) group. Values for skeletal muscle weight are the total of left and right limbs of quadriceps, gastrocnemius, and tibialis anterior. Significant differences (P<0.05) are denoted by different letters.

Table II. In vivo echocardiographic data.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>No Tumor</th>
<th>Tumor</th>
<th>Pair-Fed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart rate, bpm</td>
<td>528±8</td>
<td>418±13</td>
<td>527±8</td>
</tr>
<tr>
<td>LVDD, mm</td>
<td>3.4±0.1</td>
<td>3.2±0.1</td>
<td>3.4±0.1</td>
</tr>
<tr>
<td>LVDS, mm</td>
<td>1.6±0.1</td>
<td>2.3±0.1</td>
<td>1.6±0.1</td>
</tr>
</tbody>
</table>

Values are means ± SEM. LVDD, left ventricle (LV) internal diameter at diastole; LVDS, LV internal diameter at systole. Significant differences (P<0.05) are denoted by different letters.
increased fibrosis in the hearts of tumor-bearing mice (Fig. 3). Transmission electron microscopy revealed ultrastructural features of cardiomyocytes from tumor-bearing mice, including disrupted alignments of sarcomeric structure (disorganized I-band, M-band, A-band and Z-line structures) with bundles waving, and randomly dispersed mitochondria with a variety of sizes and shapes. Some mitochondria demonstrated impaired integrity, including broken membranes and disorganized cristae in hearts of tumor-bearing mice (Fig. 4). We were unable to detect any areas with disruptions of sarcomere structure or mitochondria in the No Tumor and Pair-fed groups.

**Composition of contractile proteins is altered in cardiac muscle of mice with cancer cachexia.** To explore whether the impaired cardiac function and structure is accompanied by altered gene expression of contractile proteins in hearts of tumor-bearing mice, we performed real-time PCR analysis for molecules with potential significance for functional performance. Gene expression of troponin I, a key myofilament protein and regulator of cardiac muscle contractility, was decreased by 38% in hearts of the Tumor mice compared to the No Tumor group (Fig. 5A). Myosin heavy chain (MHC) isoforms have ATPase activity and are a major determinate
Markers of inflammation are increased in cardiac muscle of mice with cancer cachexia. Cancer cachexia is characterized by increased levels of inflammation (26). Interleukin-6 (IL-6) plays a critical role in mice with colon-26 adenocarcinoma induced cancer cachexia (27-29). To explore the inflammatory level in the heart, real-time PCR was performed on markers of inflammation. The transcript levels of IL-6 and IL-6 receptors were increased by 5.7-fold and 2.3-fold, respectively in hearts of tumor-bearing mice compared to mice in the No Tumor group. F4/80, a marker of macrophage infiltration, was increased by 1.9-fold in the hearts of tumor-bearing mice (Fig. 6).

Discussion
This study evaluated the consequences of cancer cachexia with regard to heart function, myocardium structure, and alterations in composition of myofibrillar proteins. Our results demonstrate that cachectic mice with colon-26 adenocarcinoma tumors exhibited decreased cardiac function, which was not dependent on reduced food intake.

CD2F1 mice inoculated with colon-26 adenocarcinoma are a well-established model of cancer cachexia (27). Cancer cachexia was induced in these mice as shown by significantly lower body weight and loss of adipose and skeletal muscle masses (Table I). These results are consistent with the feature that both muscle and adipose tissues undergo rapid atrophy in this murine model for cancer cachexia (27,30). Both liver and spleen were dramatically enlarged, which is in accordance with the clinical symptoms in colorectal cancer patients that masses of tissues with a high metabolic rate, including liver, spleen and tumor are increased due to systemic inflammation and acute phase protein synthesis (31). While absolute heart weight is lower in tumor-bearing mice compared with mice in the No Tumor group, the proportion of heart relative to tumor-free body weight is higher. The higher relative weight could be attributed to edema (accumulation of fluid and inflammatory cells) considering the increased inflammation status. Inconsistent with the literature (27,32,33) we found food intake is decreased in colon-26 tumor-bearing mice. However, decreased food consumption alone is not sufficient to cause skeletal muscle wasting in this model given the fact that the weights of skeletal muscles were comparable in the Pair-fed and No Tumor groups.

To our knowledge, this is the first study to examine heart function in vivo in conjunction with evaluation of myocardium structure and myofibrillar protein composition in a mouse model of cancer cachexia. A few studies have shown evidence of decreased cardiac muscle mass and loss of contractile and myofibrillar proteins in tumor-bearing animals and cachectic patients (11,16). As for cardiac function, earlier findings suggested that deteriorations in heart function were minimal (12,13). Because of the application of transthoracic echocardiography to study left ventricle function in mice (35,36),
evaluation of heart function in vivo is accurate and efficient. Our study clearly demonstrated an impaired contractile function in colon-26 tumor-bearing mice, and this functional deterioration was not dependent on reduced food intake.

Sarcomere disorganization and mitochondrial damage are linked with hypertrophic cardiomyopathy (37) and dilated cardiomyopathy (38-40). An earlier study by Sjöström et al (12) reported smaller cross-sectional area and mitochondrial volume density of myocardial cells without severe morphological abnormality in cachectic C57BL/6J mice bearing methylcholanthrene-induced syngeneic sarcoma. In their study, no metabolic markers of myocardial function were found to be altered in tumor-bearing mice, which led the authors to reach the conclusion that myocardial function was protected in cachetic mice (12). In our study, both Masson’s trichrome staining and electron microscopy revealed structural changes in the heart of tumor-bearing mice, including increased fibrosis between myofibers, abnormal alignment of myofibers, irregular mitochondrial shape, size and dispersion, and disrupted mitochondrial structure. These changes may be a mechanism underlying the impaired contractile function detected by echocardiography. The different findings from the study of Sjöström et al (12) may be explained by different models as well as the stages of cancer cachexia that were studied. Whereas the former study was carried out at an early stage of cancer cachexia (tumor weight occupied 6-7% of carcass weight), our study was performed in terminally ill mice. These discoveries mirror a recent comment that heart failure is a slow process in cancer cachexia and may be prominent before death (6).

Cardiac failure is characterized by a switch of gene expression from ‘adult’ to ‘fetal’ isoforms in both animals and human patients (22-25). MHCα, which predominates in adult mouse hearts has higher ATPase activity than MHCβ, which predominates during embryonic development. Even a small magnitude of isoform shift could cause consequences in contractile proteins during embryonic development. Even a small magnitude of isoform shift could cause consequences in contractile proteins during embryonic development. Even a small magnitude of isoform shift could cause consequences in contractile proteins during embryonic development. Even a small magnitude of isoform shift could cause consequences in contractile proteins during embryonic development. Even a small magnitude of isoform shift could cause consequences in contractile proteins during embryonic development. Even a small magnitude of isoform shift could cause consequences in contractile proteins during embryonic development. Even a small magnitude of isoform shift could cause consequences in contractile proteins during embryonic development.

Using gel electrophoresis, we were able to confirm the induction of Tumor mice, as demonstrated by the induction of IL-6, IL-6 receptor and macrophage marker F4/80. Increased IL-6 might mediate changes in tissue protein turnover in a rat cancer cachexia model. Br J Cancer 84: 946-950, 2001.

Our data provide compelling evidence for heart function deterioration in association with cancer cachexia. This impaired function is associated with myocardium structure derangement, altered composition of myofibrillar proteins and increased inflammation. This study adds a new dimension in the understanding of the pathogenesis and consequences of cancer cachexia. Specifically, the current dogma states that fatigue and weakness in cancer cachexia can be attributed to skeletal muscle wasting. Our results support the idea that insufficient heart performance might also be responsible for the fatigue symptoms, leading to even less movement and exercise, followed by more severe muscle wasting. Therefore, a vicious cycle is developed and contributes to the morbidity of cancer cachexia. Admittedly in the current study, these cardiac abnormalities were found in cachectic mice at late stages of their lives, and it is rare to see such severe situations in clinical settings. However, it is likely that the alteration of cardiac muscle structure and fetal gene expression pattern occur early and precede the exhibited heart function deterioration. Future studies will determine the time course of these events leading to decreased heart function, and elucidate what is driving these observed cardiac abnormalities. Our previous findings suggested dysregulated lipid metabolism might be in part responsible for the ultrastructural changes of cardiac muscle (46). Further research will seek to determine whether blocking these events in early stages of cachexia using nutritional and/or pharmacological agents may prevent heart function deterioration, improve the quality of life and enhance survival for patients suffering from cancer cachexia.

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References


