Cancer Causes Cardiac Atrophy and Autophagy in a Sexually Dimorphic Manner

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Abstract

Approximately one-third of cancer deaths are caused by cachexia, a severe form of skeletal muscle and adipose tissue wasting that affects men more than women. The heart also undergoes atrophy in cancer patients, but the mechanisms and the basis for apparent sex differences are unclear. In a mouse colon-adenocarcinoma model, cancer causes a loss of cardiac mass due to a decrease in cardiac myocyte size that is associated with reduced levels of all sarcomeric proteins. Unlike skeletal muscle cachexia, atrophic hearts do not upregulate the ubiquitin-proteasome system or its activity but increase autophagy. Thus, cancer causes cardiac atrophy by a mechanism distinct from that in skeletal muscle. Male tumor-bearing mice have a more severe phenotype than females, including greater cardiac mass loss and mortality, a more robust pro-inflammatory response to the tumor, and greater cardiac autophagy. In females, estrogen protects against cancer-induced cardiac atrophy and body weight loss by signaling through its receptor. Sex differences in cardiac atrophy need to be considered during the treatment of patients suffering from chemotherapy-induced cardiomyopathy to prevent exacerbation of cardiac dysfunction. Cancer Res; 71(5); 1710–20. ©2010 AACR.

Introduction

Cachexia is a severe form of skeletal muscle and adipose tissue wasting often associated with diseases such as cancer, sepsis, and AIDS. Cachexia affects approximately one half of cancer patients and causes nearly one third of cancer deaths (1). Weight loss is due to complex alterations in carbohydrate, lipid, and protein metabolism (2). The consequences of these metabolic changes include anemia, insulin resistance, production of acute phase proteins, and a negative nitrogen balance, which cannot be reversed with nutritional supplementation. These pathologic perturbations result in a significant loss of muscle protein, leading to pronounced muscle weakness and fatigue, increased sensitivity to infections, decreased responsiveness to both chemotherapy and radiation treatment and can ultimately lead to cardiac or respiratory failure (1).

There is a significant sexual dimorphism in muscle mass loss and survival in cancer patients. Multiple studies have found that male cancer patients lose more body weight and muscle mass than females and have shorter overall survival (3, 4). Since cachexia increases mortality, it is likely that sex hormones are mediating these differences because postmenopausal women lose their survival advantage, and estrogen therapy decreases colon cancer mortality (5, 6).

Humoral factors secreted from or induced by the tumor are responsible for initiating skeletal muscle and body mass loss. Tumors induce a host immune response resulting in increased serum levels of pro-inflammatory cytokines, which cause skeletal muscle protein loss and cachexia in vitro and in vivo (7, 8). Muscle mass loss can be due to increased protein degradation, decreased protein synthesis, or both (9), but cancer cachexia is primarily due to increased proteolysis (10). Cardiac and skeletal muscles utilize 3 major proteolytic pathways: the lysosome, Ca^{2+}-dependent calpains, and the ubiquitin-proteasome system (UPS). The UPS is responsible for the bulk breakdown of long-lived proteins and plays a major role in skeletal muscle protein degradation due to cancer (11). Components of the UPS are upregulated in the muscles of cancer patients and tumor-bearing rodents and inhibition of the UPS, but not the other proteolytic pathways, suppresses tumor-induced muscle proteolysis in vitro (12, 13).

Cardiac muscle is labile and can undergo atrophy due to anorexia, prolonged bed rest, left ventricular assist device placement, and HIV (14–16). Given the high prevalence and mortality rate of cachexia and the fact that the heart is a striated muscle like skeletal muscle, it is surprising that relatively little attention has been paid to cardiac muscle atrophy in cancer patients. In 1968, Burch and colleagues observed that cancer patients had smaller hearts and decreased amplitude and duration of the QRS complex.
implying functional defects (17). Cardiac atrophy in tumor-bearing rodents has also been observed (18), but the extent of cardiac muscle atrophy, functional implications, biochemical mechanisms, and sex differences have never been fully characterized in any tumor model.

We have established a murine model of cancer-induced cardiac atrophy. Here we report that cardiac mass decreases rapidly during the course of tumor progression and there are multiple, significant differences in the disease phenotype between males and females. Males lose more body weight, skeletal, and cardiac muscle than females and have a worse phenotype in all cardiac parameters we studied. We also show that cardiac atrophy is due to a decrease in all myofibrillar proteins, as opposed to myosin heavy chain (MyHC) specifically as reported in skeletal muscle cachexia (7). Most importantly, we provide data implicating autophagy as the main proteolytic pathway involved. To our knowledge, this report provides the first insight into this previously unappreciated aspect of cancer cachexia in both sexes and shows that the mechanisms of cardiac muscle atrophy are distinct from those in skeletal muscle.

**Materials and Methods**

**Animals**

CD2F1 (Balb/c X DBA2) is the mouse strain used in all cachexia studies. Colon-26 adenocarcinoma (C-26; 5 x 10^5 cells in 100 μL PBS) was injected subcutaneously into the right flank of 8-week old male and female mice. Echocardiography was done as previously described (19). Serum from control and tumor-bearing animals was analyzed with a cytokine multiplex panel (Milliplex; Millipore). Fulvestrant (ICI 182,780; Sigma) was dissolved in 70% ethanol and Cremaphor EL. Female mice were injected subcutaneously with 5 mg of ICI once weekly for 4 weeks starting the day of tumor cell inoculation. All animal studies were reviewed and approved by the Institutional Animal Care and Use Committee at the University of Colorado at Boulder.

**Immunohistochemistry and histology**

Hearts were fixed in 10% buffered formalin and processed by Premier Laboratory. Slides were deparaffinized and stained with Texas red-X conjugated wheat germ agglutinin (WGA) or anti-cathepsin D (20) and analyzed with a Nikon Eclipse E800 microscope. For fibrosis analysis, slides were stained with Picrosirius Red, analyzed with polarized light microscopy (Zeiss Universal Microscope), and quantified with ImageJ.

**Western blots and myofibrillar gels**

For MyHC analysis, left ventricles were homogenized in high salt myosin extraction buffer (21). For other proteins, left ventricles were homogenized in 50 mmol/L Tris pH 7.5, 150 mmol/L NaCl, 50 mmol/L NaF, 1 mmol/L EDTA, 0.5% Triton X-100 (1% Triton for LC3 analysis), and complete protease inhibitor cocktail (Roche). MyHC antibodies were: α-MyHC (BA-G5 hybridoma, ATCC), and anti-β-MyHC (VP-M667, Vector Laboratories). Other antibodies were: anti-ubiquitin (Santa Cruz), caspase-9, LC3B, and GAPDH (glyceraldehyde 3-phosphate dehydrogenase; Cell Signaling Technology). Cardiac myofibrils were purified as previously described (22). Gels were loaded with equivalent percent of final heart weight (0.005%), stained with colloidal Coomassie Blue (ProtoBlue Safe; National Diagnostics), and analyzed with ImageJ.

**RNA analysis**

Total RNA was purified using TRI Reagent (Ambion) according to the manufacturer's protocol. cDNA was synthesized with Superscript II reverse transcriptase (Invitrogen) and random hexamer primers. Gene expression was determined by quantitative real-time reverse transcriptase PCR (qRT-PCR) using SYBR Green dye with gene specific primer sets and an Applied Biosystems 7500 Real-Time PCR system.

**Caspase-3 and proteasome activity assays**

Caspase-3 activity in cardiac lysates was determined as described (19). Proteasome activity assays were carried out as described (23) with Suc-LLVY-AMC (Boston Biochem) as the substrate. Cleavage data were obtained over 1 hour and activity was determined by calculating the slope of the linear portion of the graph. Bortezomib (Velcade) was kindly provided by Millennium Pharmaceuticals.

**Ubiquitin conjugation assay**

Briefly, left ventricular tissue homogenate (1 mg/mL) was incubated with 2 μg myc-Ub, 2 mmol/L Energy Regeneration Solution, 50 μmol/L MG-132, and 1 μmol/L ubiquitin aldehyde (all from Boston Biochem) at 37°C for 30 minutes. The reaction was quenched with SDS-PAGE sample buffer. Ubiquitin-protein conjugates were detected by an anti-myc antibody (Cell Signaling).

**Electron microscopy**

Electron microscopy was carried out as described (20). Hearts were retrograde perfused with 2% gluteraldehyde in 0.1 mol/L cacodylate buffer. Hearts were then postfixed in 2% osmium tetroxide and 1% uranyl acetate for 1 hour each. Tissue was dehydrated using a series of ethanol washes, rinsed in propylene oxide and 1% uranyl acetate for 1 hour each. Tissue was then placed into Epon resin overnight, and polymerized at 60°C for 48 hours. Sections were cut with a Diatome Ultra diamond knife, poststained in 2% uranyl acetate and Reynolds’s lead citrate, and viewed using a Philips CM100 electron microscope.

**Data and statistical analysis**

Data are presented as mean ± SEM. Differences between groups were evaluated for statistical significance using Student’s t test. P values less than 0.05 were considered significant.
Results

Cardiac atrophy caused by colon-26 adenocarcinoma is more pronounced in males than in females

In order to define cardiac atrophy due to cancer and to determine whether there are sex differences, male and female mice were inoculated subcutaneously with colon-26 adenocarcinoma (C-26) cells, which cause a well-characterized cancer cachexia, resulting in rapid and severe skeletal muscle atrophy that is independent of anorexia (24). Male mice lost significantly more body weight than females at all time points studied (Fig. 1A). Both male and female mice lost significant skeletal muscle mass at 15 days, and continued to lose muscle mass along the course of disease. The rate and extent of muscle mass loss was significantly greater in males than females (Fig. 1B).

We measured cardiac mass at 15, 21, and 27 days post-tumor cell inoculum. Male mice lost 8.3% of their cardiac mass at 15 days, which progressed to 21.8% at 27 days (Fig. 1C). Interestingly, female mice initially gained cardiac mass and lost only 9.8% by day 27. Males lost significantly more cardiac mass than females at days 15 and 27. The rate at which cardiac mass was lost also differed between the sexes (Fig. 1C). This sexual dimorphism cannot be attributed to differences in tumor mass (Fig. 1D). In addition to having less cardiac atrophy, female tumor-bearing mice survived about 1 week longer than males (data not shown).

Since pro-inflammatory cytokines contribute to skeletal muscle cancer cachexia (8), we conducted a Multiplex ELISA on male and female mouse serum at days 15 and 27. We found that males had a trend toward higher levels of pro-and anti-inflammatory cytokines than females (Supplementary Fig. 1 and Supplementary Table 1), which correlates with findings in colorectal cancer patients (25). The enhanced inflammatory profile in male tumor-bearing mice may therefore contribute to the increased cardiac and skeletal muscle mass loss and decreased survival we observed.

Estrogen signaling is required for the maintenance of cardiac muscle mass in female tumor-bearing mice

Female cancer patients lose less skeletal muscle and body mass than men (3). Because we observed this in our mouse model, we determined if estrogen plays a role in the prevention of cardiac muscle mass loss in females by injecting female mice with Fulvestrant (ICI 182,780), a potent and specific
estrogen receptor (ER) antagonist (26). Fulvestrant did not affect either cardiac mass or body weight in non–tumor-bearing mice. Fulvestrant treatment did not affect cardiac mass (A) or body weight (BW; C) in control mice. Inhibition of estrogen signaling in tumor-bearing females results in equivalent cardiac mass (B) and body weight (D) loss as tumor-bearing males. Percent loss in ICI group was calculated relative to the non-ICI controls. Heart weight (HW) is normalized to tibia length (TL) to normalize for mouse age and size. n = 6 per group. Graphs are mean ± SEM. **, P < 0.01; ***, P < 0.0001.

Figure 2. ER signaling is required for the maintenance of cardiac and body mass in female tumor-bearing mice. Fulvestrant treatment does not affect cardiac mass (A) or body weight (BW; C) in control mice. Inhibition of estrogen signaling in tumor-bearing females results in equivalent cardiac mass (B) and body weight (D) loss as tumor-bearing males. Percent loss in ICI group was calculated relative to the non-ICI controls. Heart weight (HW) is normalized to tibia length (TL) to normalize for mouse age and size. n = 6 per group. Graphs are mean ± SEM. **, P < 0.01; ***, P < 0.0001.

Atrophic hearts have increased fibrosis and decreased aortic pressure and velocity

Cardiac fibrosis is often implicated in cardiac pathology and contributes to decreased function (27). In order to determine the extent of fibrosis in atrophic hearts, transverse sections were stained with Picrosirius Red, which reveals myocellular disarray and collagen deposition. We found that both male and female atrophic hearts had significant increases in fibrosis (50% and 65%, respectively) and significant myocellular disarray (Fig. 3A). Hearts from both control and tumor-bearing females had less (22% and 17%, respectively) fibrosis than males but the fold change in fibrosis due to cancer was not significantly different between the sexes. Because collagen deposition and myocellular disarray cause myocardial stiffness and a decrease in cardiac function (28), we conducted M-mode echocardiography on male and female mice at day 27. Male atrophic hearts had a significant 30% decrease in aortic pressure and a 16% decrease in aortic velocity (Fig. 3B).

Female tumor-bearing mice, however, did not have a decrease in any of the functional parameters studied (Fig. 3B and Supplementary Table 2). Surprisingly, neither ejection fraction nor fractional shortening changed in either sex despite the extensive cardiac muscle loss.

Cardiac atrophy is due to a decrease in myocyte size and not an increase in cell death

Cancer-induced skeletal muscle atrophy is primarily due to increased protein degradation resulting in decreased myofiber size (10). Though apoptosis does occur in cachectic skeletal muscle, it does not significantly contribute to muscle mass loss (29). In order to determine how cardiac muscle atrophies in our mouse model, we first quantified myocyte size in male and female control and atrophic hearts (day 27). Cardiac myocyte cross-sectional area from male tumor-bearing mice was 31% smaller than male controls, while atrophic female myocytes were only 16% smaller than female controls (Fig. 4A). The sexually dimorphic decrease in myocyte area correlates with the sex difference in cardiac mass loss.

To determine if increased cell death also plays a role in cardiac atrophy in males, we measured caspase-3 activity and the levels of caspase-9 cleavage products in cardiac muscle extracts and did not find an increase in either of these apoptotic markers at day 15 or 27 (Fig. 4B and C). The amount of DNA per mg of tissue also did not change in atrophic cardiac muscle, while the amount of protein per mg tissue significantly decreased, as expected (Fig. 4D). Together, these
results indicate that cardiac muscle atrophy is due to a decrease in cell size, rather than an increase in apoptosis.

**All sarcomeric proteins are equally decreased in atrophic cardiac muscle**

Myofibrillar proteins make up 40% of left ventricular dry weight (30). Therefore, a decrease in myocyte size must be accompanied by a decrease in myofibrillar proteins. When the same percent of final heart weight was analyzed, we found that male atrophic hearts contained 22% less MyHC than controls (Fig. 5A). Contrary to previous studies in skeletal muscle (7), we also found a significant decrease in sarcomeric actin that paralleled the decrease in MyHC (Fig. 5A and Supplementary Fig. 2A). MyHC/actin ratios therefore did not change, which suggests that entire cardiac sarcomeres are degraded during cancer-induced atrophy. To determine if all sarcomeric components decrease in parallel, we purified myofibrils from male atrophic and control hearts. When the same percent of final heart weight was loaded, we found a parallel decrease in all myofibrillar components (Fig. 5B). Therefore, the absolute amounts of myofibrillar proteins decreased but the ratio of myofibrillar proteins in the sarcomere was maintained, presumably to preserve cardiac function.

Cardiac muscle contains 2 MyHC isoforms: α and β. The murine heart is primarily composed of α-MyHC, which has faster ATPase kinetics than β (31). A small increase in β-MyHC can cause decreased Ca2+-activated ATPase activity and systolic function (32) and is a marker of pathology. qRT-PCR revealed that β-MyHC mRNA expression increased in both sexes at day 15, and significantly increased 16-fold in females and 22-fold in males at day 27 (Fig. 5C). α-MyHC mRNA levels did not change in males but significantly increased 3-fold in females at day 15, which correlates with the sex difference in cardiac mass at that time. In contrast to mRNA data, western blots revealed that α-MyHC protein decreased starting at day 15 and continued to decrease to 70% of control levels at day 27 in male hearts (Fig. 5D). These results indicate that cardiac MyHCs are posttranscriptionally regulated, and suggest that increased protein degradation is responsible for the observed atrophy. Importantly, there was a significant increase in β-MyHC protein (Fig. 5D and Supplementary Fig. 2B), which is considered pathologic in the rodent heart.

**The UPS is not upregulated in cancer-induced cardiac atrophy**

Skeletal muscle atrophy is accompanied by increases in transcription of a common, specific set of genes that are involved in protein degradation by the UPS (33). Atrogin-1 and MuRF-1, muscle-specific E3 ubiquitin ligases, did not
increase in male or female atrophic hearts, while expression increased 8-and 11-fold, respectively, in the gastrocnemius of the same male animals (Fig. 6A and B). Interestingly, the heart had 1.5- and 3-fold higher levels of atrogin-1 and MuRF-1 transcripts, respectively, than skeletal muscle and males had higher levels of these transcripts than females (Supplementary Fig. 3A and B). Because the UPS is primarily responsible for skeletal muscle protein degradation (34), we quantified the levels of polyubiquitinated proteins in both the soluble and insoluble fractions of male cardiac muscle lysates and did not find significant differences at any time point (Fig. 6C). Cachectic skeletal muscle has increased ubiquitination activity (35), but we did not find a change in ubiquitin conjugation activity in atrophic cardiac muscle at day 15 or 27 (Fig. 6C). Cachectic skeletal muscle has increased ubiquitination activity (35), but we did not find a change in ubiquitin conjugation activity in atrophic cardiac muscle at day 15 or 27 (Fig. 6C). Additionally, proteasome activity in atrophic cardiac muscle lysates did not change at day 15 or 27, while it increased 2-fold in the gastrocnemius (Fig. 6D). Together, these results indicate that unlike skeletal muscle, UPS activity is not upregulated in the hearts of tumor-bearing mice.

Autophagy is upregulated in atrophic hearts

Autophagy, a mechanism by which cells degrade large quantities of intracellular protein during periods of cellular stress, has been shown to play a larger role in the heart than in skeletal muscle (36). Cathepsin L, beclin, and LC3 (microtubule-associated protein 1 light chain 3) are well-characterized markers of increased lysosomal activation during myocyte atrophy and autophagy (37). Cathepsin L mRNA significantly increased 2-fold in both male and female atrophic hearts, while LC3 mRNA increased approximately 1.5-fold at day 27 (Fig. 7A). Cytosolic LC3-I is lipidated to form LC3-II upon activation of autophagy. LC3-II is the only well-characterized protein that is specifically localized to autophagic vacuoles and serves as an accurate marker for autophagy (38). LC3-II protein levels were 7-fold higher in male atrophic hearts and only 3-fold higher in females at days 15 and 27 (Fig. 7B). LC3-II was significantly higher in male than female hearts at day 27, which could explain the increased cardiac mass loss in tumor-bearing males. Direct evidence of autophagy was obtained by electron microscopy, which revealed the presence of...
numerous double-membraned autophagic vacuoles that contained portions of cytoplasm, mitochondria, and myelin-like structures (Fig. 7C and Supplementary Fig. 4B). Autophagic vacuoles were rarely detected at day 15, but were abundant by day 27. Additionally, atrophic hearts stained more heavily for cathepsin D, a lysosomal protease (Supplementary Fig. 5). These results show that autophagy increases along the course of disease in cardiac muscle of tumor-bearing mice and is likely playing a major role in the enhanced protein degradation responsible for cardiac muscle atrophy.

Discussion

More than one half of cancer patients suffer from cachexia, a severe muscle-wasting syndrome that results in decreased prognosis and survival (1). Cancer also causes cardiac muscle atrophy, a phenomenon that has been understudied by the scientific and oncology communities. A recent study found a direct correlation between muscle mass loss and death, and postulated that cardiac muscle atrophy contributed to the decreased survival in tumor-bearing mice (39). Our studies reveal the phenotype, mechanisms and sex differences of cardiac atrophy and show that it is distinct from skeletal muscle. Our results show that cardiac atrophy is an important feature of cancer cachexia that should be considered during the treatment and management of cancer patients, particularly in the setting of chemotherapy-induced cardiotoxicity or pre-existing heart disease.

We have shown that atrophic hearts had significantly decreased levels of all myofibrillar proteins. The issue whether sarcomeric proteins are selectively depleted is controversial; some have shown a specific decrease in MyHC (7), while others report that MyHC is not selectively lost in muscle atrophy (22). Although we show a parallel decrease in all sarcomeric proteins in the later stages of atrophy, it is possible that MyHC is specifically decreased early in cardiac atrophy, before any measurable mass loss. Presumably, absolute decreases in the number of sarcomeres would result in decreased cardiac function, which is why we were surprised to find that neither ejection fraction nor fractional shortening changed in either sex. However, other models of cardiac atrophy have also shown that atrophic hearts maintain their ejection fraction (40, 41), implying that cardiac muscle is able to adapt functionally to muscle protein loss up to a certain point. We did find that male tumor-bearing mice had a significant decrease in aortic pressure and velocity, which correlates with clinical findings of hypotension in cancer patients.

The increases in β-MyHC we observed in the atrophic heart could have significant functional implications that may not be detected by echocardiography. β-MyHC induction in the rodent heart is pathologic and can contribute to myocardial contractile dysfunction since cardiac pressure development and power output decrease as β-MyHC increases (32, 42). A more gradual model of cardiac atrophy might reveal higher levels of β-MyHC than we observed, which could affect cardiac

Figure 5. All sarcomeric proteins are decreased in atrophic hearts. A, Coomassie Blue–stained gel (0.005% final cardiac mass) shows MyHC and actin levels decrease in parallel in atrophic hearts. B, SDS–PAGE of purified myofibrils revealed that all sarcomeric proteins decrease in parallel during cardiac atrophy. Samples were run on the same gel but were noncontiguous. C, fold change of MyHC mRNA (normalized to 18S expression) in each sex compared with controls. D, α- and β-MyHC protein expression in males at day 27. MyHC was solubilized in a high salt buffer and 0.5 μg was loaded. It was therefore not possible to reprobe the blot for a loading control. Mean ± SEM. *, P < 0.05; **, P < 0.01; and ***, P < 0.0001 versus control. n = 4–5 per group.
function. Since cachexia in our mouse model is very rapid and leads to death in approximately 4 weeks, there may have been decreased cardiac function if the mice had lived longer. Because most cancer patients live with a tumor burden for many years, it is possible that cardiac abnormalities occur but likely remain undiagnosed due to insufficient monitoring.

Figure 6. Atrogin-1 and MuRF-1 expression levels do not change in atrophic hearts, but are significantly upregulated in atrophic skeletal muscle. A, atrogin-1 and MuRF-1 mRNA fold changes (normalized to 18S expression) in male and female control and atrophic hearts. B, relative atrogin-1 and MuRF-1 gene expression at day 27 in male heart and skeletal muscle (gastrocnemius). C, Western blot of day 15 cardiac muscle lysates for total ubiquitin (Ub). Bottom, ubiquitin conjugation assays do not show a difference in ubiquitination activity between groups. D, proteasome activity in atrophic cardiac and skeletal muscle. Bortezomib is a potent and specific proteasome inhibitor. $n = 4$ per group. Mean ± SEM. *, $P < 0.05$; **, $P < 0.01$ versus control.
Figure 7. Autophagy is upregulated in male and female atrophic hearts and is nearly 3-fold higher in males. A, fold change of autophagy marker transcripts (normalized to 18S) in male and female atrophic hearts at days 15 and 27. B, Western blots revealed increases in LC3-II levels in the hearts of both male and female tumor-bearing mice, though it was only significant in males. Quantification represents the band density of blots containing males and females on the same gel (not shown). n = 4 per group. C, electron micrographs from the left ventricle of tumor-bearing males (day 27). Atrophic hearts contain numerous autolysosomes and double-membraned autophagosomes (arrows) containing cellular components. Mean ± SEM. *, P < 0.05 and **, P < 0.01 versus control. §, significance compared with males.
The proteolytic pathways involved in skeletal muscle cachexia have been well characterized and we were not expecting to find differences in the pathways upregulated in cardiac muscle. The UPS mediates skeletal muscle atrophy, but we did not find an increase in total ubiquitinated proteins, ubiquitin conjugating activity, or proteasome activity in atrophying cardiac muscle. The heart may not upregulate the UPS because it already has a high basal activity: cardiac muscle has a higher metabolic rate than skeletal muscle and has a higher protein turnover rate (43). Accordingly, expression of UPS components and proteasome activity are greater in cardiac than skeletal muscle (44). The baseline levels and activity of UPS components in the heart may therefore be sufficient to process the increased supply of substrates during cardiac atrophy.

Autophagy plays only a minor role in skeletal muscle atrophy (13), but we found a significant increase in autophagy in the hearts of tumor-bearing mice. Autophagy is essential for cardiac homeostasis and cardiac-specific reduction of autophagy results in contractile dysfunction, increased levels of polyubiquitinated proteins and increased apoptosis (45), indicating that autophagy is important for protein turnover and clearance of misfolded or aggregated proteins. In fact, cardiac autophagy is induced in response to intracellular protein aggregates (46) and it is thought to be beneficial by removing aggregates that are unable to be cleared by the UPS. In addition to degrading mitochondria, it is possible that autophagosomes also degrade myofibrillar proteins cleaved from the sarcomere during cardiac atrophy in order to prevent protein aggregation and to preserve cardiac function. Although there is evidence for lysosomal degradation of myofibrillar proteins (47), it is a very controversial issue, and will be an important area of future investigation.

Cachexia increases mortality in both humans and rodents (39, 48). Interestingly, male cancer patients have more severe cachexia and increased mortality than females (3, 49). Accordingly, we found striking sex differences in cardiac and body mass loss and in all of our molecular analyses. We showed that ER signaling protects females against body weight and cardiac mass loss, indicating that estrogen could also be involved in the increased survival we observed in females. The increase in body mass loss we observed with Fulvestrant treatment must be due to increased fat loss since skeletal muscle mass was not affected. Female ERα knockout mice have increased skeletal muscle mass (30), which indicates that muscle mass is not positively regulated by ER signaling. Although decreases in estrogen are typically known to increase fat mass, the role of estrogen in regulating fat mass in a cachexia model is unknown, and will be an interesting area of future study. Collectively, these studies offer much-needed insight into the effects of cancer on the heart, and suggest that cardiac function in cancer patients, particularly in males, requires closer monitoring.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

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