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Mitochondria-Related Gene Expression Changes Are Associated With Fatigue in Patients With Nonmetastatic Prostate Cancer Receiving External Beam Radiation Therapy

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Abstract

Background—Cancer-related fatigue (CRF) is associated with negative health outcomes and decreased health-related quality of life; however, few longitudinal studies have investigated molecular-genetic mechanisms of CRF.

Objective—The objective of this study was to describe relationships between mitochondria-related gene expression changes and self-reported fatigue in prostate cancer patients receiving external beam radiation therapy (EBRT).

Methods—A prospective, exploratory, and repeated-measures design was used. Self-report questionnaires and peripheral whole-blood samples were collected from 15 patients at 7 time points. Baseline data were compared against 15 healthy controls. The Human Mitochondria RT² Profiler PCR Array was used to identify differential regulation of genes involved in mitochondrial biogenesis and function.

Results—Compared with baseline, there were significant increases in fatigue scores ($P = .02-.04$) and changes in mitochondria-related gene expression ($P = .001-.05$) over time. Mean fatigue scores were 1.66 (SD, 1.66) at baseline, 3.06 (SD, 1.95) at EBRT midpoint, 2.98 (SD, 2.20) at EBRT completion, and 2.64 (SD, 2.56) at 30 days after EBRT. Over time, 11 genes related to mitochondrial function and structure were differentially expressed. Of these 11 genes, 3 (*BCL2L1*, *FIS1*, *SLC25A37*) were more than 2.5 fold up-regulated, and 8 (*AIFM2*, *BCL2*, *IMMP2L*, *MIPEP*, *MSTO1*, *NEFL*, *SLC25A23*, *SLC25A4*) were greater than 2-fold down-regulated. Furthermore, 8 genes (*AIFM2*, *BCL2*, *FIS1*, *IMMP2L*, *MSTO1*, *SLC25A23*, *SLC25A37*, *SLC25A4*) were significantly associated with the changes in fatigue scores.

Conclusion—This study provides preliminary evidence that 8 mitochondrial function genes were significantly associated with fatigue in prostate cancer patients during EBRT.

Implications for Practice—These findings identify possible pathways and early biomarkers for targeting novel interventions for CRF.

Keywords

Fatigue; Gene expression; Mitochondria; Prostate cancer; Radiation therapy

More than 40% of patients with cancer receive radiation therapy as part of their treatment regimen.¹ Localized external beam radiation therapy (EBRT), using an intensity-modulated radiation technique (IMRT), is a standard treatment option for nonmetastatic prostate cancer.² Although EBRT has increased survival rates for these men,³ fatigue is frequently reported.^{4,5} Cancer-related fatigue (CRF) negatively impacts health outcomes, leading to increased depression, impaired cognitive function, increased sleep disturbance, and decreased health-related quality of life.⁶⁻⁸

Cancer-related fatigue is defined by the National Comprehensive Cancer Network as a distressing, persistent, subjective sense of tiredness or exhaustion related to cancer or cancer treatment that is not proportional to recent activity and interferes with usual functioning.⁹ Ionizing radiation-induced damage is associated with many adverse effects including fatigue, nausea, vomiting, diarrhea, peripheral neuropathy, and cognitive function impairment. The presence of multiple adverse effects limits therapeutic success because of a necessary reduction in dosage and frequency of treatment.¹⁰⁻¹² Underlying etiologic mechanisms involved in radiation-induced fatigue remain unclear, making targeted therapy difficult.^{1,13} Etiologic explanations proposed for radiation-induced fatigue include a decrease in adenosine triphosphate (ATP) utilization and a decline in neuromuscular efficiency.^{13,14} These mechanisms suggest that impairment of mitochondrial function may be involved in the development of radiation-induced fatigue.

External beam radiation therapy induces apoptosis, a process of regulated cell death, within cancer cells. We hypothesize that EBRT affects the apoptotic pathway within mitochondria of nontumor cells, altering energy production and contributing to fatigue. Radiation-related damage induces cellular responses, which involve diverse pathways, including DNA damage processing, inhibition of signal transduction, mutations, altered gene expression, cell-cycle arrest, genomic instability, carcinogenesis, and cell death.^{15,16} Mitochondria are vulnerable to reactive oxygen species, which are one of the major direct causes of ionizing radiation-induced damage.¹⁰ Radiation-induced damage may alter mitochondrial structure and function by initiating the mitochondrial/intrinsic pathway of apoptosis.^{17,18} Few longitudinal studies have investigated molecular-genetic mechanisms of CRF in prostate cancer patients.¹⁹⁻²¹ This is the first study to explore the relationship between fatigue and radiation-induced changes in mitochondria-related gene expression in men with nonmetastatic prostate cancer before, during, and after EBRT.

Methods

This exploratory study used a prospective, repeated-measures design to assess fatigue in men with nonmetastatic prostate cancer prior to EBRT at 7 time points: day 0 (baseline), day 1 of EBRT, day 7, day 14, days 19 to 21 (midpoint of EBRT), days 38 to 42 (completion of EBRT), and days 68 to 72 (30 days after EBRT). Baseline data obtained from study subjects (n = 15) were compared with data obtained from age-, gender-, and race-matched controls

without prostate cancer (n = 15) for a total of 30 subjects. The use of matched controls at baseline is to demonstrate that expression of mitochondria-related genes is similar between healthy controls and prostate cancer subjects before receiving EBRT. The gene expression values of prostate cancer subjects at baseline are used as the true control for this study.

Participants and Procedures

The study (NCT00852111) was approved by the Combined Neurosciences Institutional Review Board of the National Institutes of Health (NIH), Bethesda, Maryland. Recruitment and data collection processes were conducted at the Hatfield Clinical Research Center, NIH, from May 2010 to January 2011. The study was conducted in accordance with the Department of Health and Human Services' policy for protection of human research subjects.²²

Eligible patients were referred and screened by urology and radiation oncology collaborators at the Hatfield Research Center, NIH. Informed consents were obtained from all participants. Inclusion criteria included males 18 years or older, with clinical diagnosis of localized prostate cancer, scheduled to receive EBRT using IMRT technique, concurrently receiving androgen deprivation therapy (ADT), and able to provide written informed consent. Patients were ineligible if they had progressive or unstable disease of any body system causing clinically significant fatigue; systemic infections (eg; human immunodeficiency virus, active hepatitis); documented history of major depression, bipolar disorder, psychosis, or alcohol dependence/abuse within the past 5 years; uncorrected hypothyroidism or anemia; second malignancies; or concurrent chemotherapy with their EBRT and those with chronic inflammatory disease that may alter proinflammatory cytokine profiles (eg, rheumatoid arthritis, systemic lupus erythematosus, cirrhosis). Additionally, patients taking sedatives, steroids, or nonsteroidal anti-inflammatory agents were excluded because these medications are known to affect immunogenetic changes.^{23,24}

Measures

Demographic and clinical characteristics of study participants (eg, age, ethnicity, stage of disease, prostate-specific antigen, testosterone, hematocrit, and albumin values) were retrieved by chart review. Data on demographic and clinical characteristics were collected once at baseline. Participants were screened for depression using the Hamilton Depression Rating Scale (HAM-D), a 21-item, clinician-rated paper questionnaire with good internal reliability ($\alpha = .81-.98$).²⁵ Data were collected on the HAM-D at each of the 7 time points. The predefined cutoff score for depression is 15 in cancer patients, with higher scores indicating more symptoms of depression.²⁶

FATIGUE SCORE—Fatigue was measured by the validated revised Piper Fatigue Scale, which is a 22-item paper-pencil, self-administered questionnaire that measures 4 dimensions of fatigue (behavioral/severity, sensory, cognitive/mood, and affective) using a 0- to 10-point intensity rating scale (0 = none; 10 = worst intensity). Data were collected at each of the 7 time points. Scores were categorized as mild fatigue (1–3), moderate fatigue (4–5), and severe fatigue (>6). The revised Piper Fatigue Scale has demonstrated satisfactory reliability and validity when used in cancer patients receiving radiation therapy with internal

consistency ranging from 0.69 for the symptom dimension to 0.95 for the sensory dimension.²⁷

GENE EXPRESSION IN PERIPHERAL BLOOD—Peripheral blood samples (2.5 mL) were collected from each subject at each of the 7 time points using PAXgene blood RNA tubes (PreAnalytiX, Hombrechtikon, Switzerland) to explore changes from baseline in gene expression related to mitochondrial biogenesis and function. The collection tubes with peripheral white blood cells were inverted 10 times to ensure red blood cell lysis immediately after collection, and the samples were immediately stored at -80°C freezer until RNA extraction. RNA extractions were processed by a single laboratory technician following a standard protocol to minimize nonbiological technical bias. Total RNA extraction, cDNA synthesis, amplification, and data analyses were performed according to manufacturer's procedure. Total RNA was isolated and extracted from frozen whole-blood samples following the PAXgene blood RNA kit procedure (PreAnalytiX). RNA yields were 3 ng or greater from each 2.5 mL of whole blood collected. All extracted RNA was purified using RNeasy mini kit (Qiagen, Valencia, California). Total RNA concentration, purity, and integrity were tested using the NanoDrop (ND-1000; Wilmington, Delaware) and Experion systems (Biorad, Hercules, California). Following RNA preparation, the samples were treated with DNase to ensure elimination of genomic DNA. A total of 100 to 150 ng of extracted RNA per sample was then converted to cDNA using the RT² First Strand Kit (SABiosciences, Frederick, Maryland). After cDNA synthesis reaction, the cDNA was diluted using nuclease-free H₂O and immediately stored at -20°C until used for human mitochondria-related gene expression profiling.

REAL-TIME POLYMERASE CHAIN REACTION ARRAY FOR MITOCHONDRIA-RELATED GENE EXPRESSION—As an exploratory study, the Human Mitochondria RT² Profiler PCR Array System (PAHS-087A; SABiosciences) was selected to evaluate expression of genes related to mitochondrial biogenesis and function. The genes evaluated in this polymerase chain reaction (PCR) array include 10 groups of mitochondrial regulators and mediators related to membrane polarization and potential, mitochondrial transport, small molecule transport, targeting proteins to mitochondria, mitochondrial protein import, outer membrane translocation, inner membrane translocation, mitochondrial fission and fusion, mitochondrial localization, and mitochondrial apoptotic genes (<http://www.sabiosciences.com/genetable>; SABiosciences). Diluted first-strand cDNA was mixed with 2× SABiosciences RT² qPCR master mix (SABiosciences). Ten nanoliters of PCR cocktail was added to each well of the 384-well PCR array for real-time PCR detection. The real-time PCR was carried out using the ABI PRISM 7900HT Real-Time PCR System. SYBR green fluorescence was detected from each well during the annealing step of each cycle through the real-time thermal cycler program.

Statistical Analyses

Descriptive statistics were used to describe demographic/clinical characteristics of sample, fatigue scores, and changes in gene expression at each time point. An independent *t* test was used to compare differences in fatigue scores and changes in gene expression between patients and controls at baseline. A mixed linear effects model was used to describe the

changes in gene expression and fatigue scores over time and to determine the association between changes in each gene expression and fatigue scores at each time point. Statistical analyses were conducted using the Statistical Analysis System version 9.3 (SAS Institute Inc, Cary, North Carolina). Power analysis was calculated using a study that reported significant difference in fold changes of gene expression and fatigue scores among breast cancer survivors²⁸ and suggested a minimum of 10 subjects were needed to obtain 90% power or greater at $P < .05$ significance level.

Polymerase chain reaction data were analyzed using the C_t method (PCR Array Data Analysis Web portal: <http://www.sabiosciences.com/pcrarraydataanalysis.php>; SABiosciences Corp, Qiagen). At least 3 reference genes (*RPL13A*, *GAPDH*, *ACTB*) were selected for normalization of data. Genes with more than a 2-fold change in gene expression and nonadjusted $P < .05$ at any time point during EBRT were considered as significant up- or down-regulation in gene expression for presentation. Additionally, to control for multiple comparisons of 84 genes, the Bonferroni-adjusted P values were calculated (P value/84). For Ingenuity pathway analysis (IPA), genes with more than a 1.5-fold change and $P < .05$ at day 14, 21, 42, or 72 were included. Because of the exploratory nature of this study, a significance level of .05 was used for determining significant up- or down-regulated genes and for selection of genes for inclusion in IPA.

Results

Sample Demographics

Fifteen patients with nonmetastatic prostate cancer undergoing EBRT and 15 age-, gender-, and race-matched controls were enrolled in the study (Table 1). The mean age of the subjects (62.8 [SD, 8.6] years) was within ± 5 years from the mean age of matched controls (57.2 [SD, 7.6] years). More than half ($n = 9/15$) of the participants had stage T2 (a–c) prostate cancer with Gleason scores (range, 6–9) and baseline prostatespecific antigen levels (range, 0.61–111 $\mu\text{g/L}$) that were consistent with intermediate- to high-risk progression of the disease.²⁹ All participants were receiving neoadjuvant therapy with ADT 8 weeks before starting EBRT. Baseline thyroid-stimulating hormone (mean, 1.8 [SD, 1.2] $\mu\text{IU/mL}$), testosterone (mean, 243.9 [SD, 159.9] ng/dL), albumin (mean, 4.1 [SD, 0.3] g/dL), and hematocrit (mean, 40.3 % [SD, 3.8%]) were within reference range (thyroid-stimulating hormone = 0.4–4.0 $\mu\text{IU/mL}$, testosterone = 181–758 ng/dL , albumin = 3.7–4.7 g/dL , hematocrit = 40.1%–51%, respectively). None of the participants reached the cutoff score for depression (HAM-D) either at baseline or at the end of EBRT. Of the 15 subjects with prostate cancer, 13 (87%) received a total of 42 fractions with 75.6 Gy using the IMRT technique.

Fatigue Score

Mean fatigue score was 1.66 (SD, 1.66) at baseline (pre-EBRT) for patients and 0.67 (SD, 1.2) for controls. There was no significant difference in fatigue scores at baseline between patients and controls ($P = .09$). The mean fatigue score increased to 3.06 (SD, 1.95) at midpoint of EBRT, slightly decreased to 2.98 (SD, 2.20) at completion of EBRT, and remained slightly elevated at 30 days after EBRT 2.64 (SD, 2.56). However, there was a

significant change in fatigue score over time during EBRT compared with baseline data ($P = .001-.04$) for the sample. Figure 1 illustrates fatigue score changes over time for each of the 15 participants. Subset analysis of fatigue scores showed that, of the 15 subjects, 6 had more than a 3-point change in fatigue scores from baseline to day 21, 7 had more than a 3-point change from baseline to day 42, 9 had less than a 3-point change from baseline to day 21, and 8 had less than a 3-point change from baseline to day 42. The 3-point change in fatigue scores is noted to be clinically significant using the Piper Fatigue Scale.³⁰

Mitochondria-Related Gene Expression

There was no significant difference in mitochondria-related gene expression at baseline between patients and controls ($P = .07-.86$). Eleven genes related to mitochondrial function were differentially expressed over time during EBRT compared with baseline ($P < .05$). Three of the 11 genes (*BCL2L1*, *FIS1*, *SLC25A37*) were greater than 2.5-fold up-regulated (Figure 2), and 8 of the 11 genes were greater than 2-fold down-regulated. These 8 down-regulated genes included the apoptosis-inducing factor mitochondrion associated 2 (*AIFM2*), B-cell CLL/lymphoma 2 (*BCL-2*), IMP 2 inner mitochondrial membrane peptidase-like (*IMMP2L*), mitochondrial intermediate peptide (*MIPEP*), Misato homolog 1 (*Drosophila*) (*MSTO1*), neurofilament, light polypeptide (*NEFL*), solute carrier family 25-member 23-mitochondrial carrier, phosphate carrier (*SLC25A23*), and solute carrier family 25-member 4-mitochondrial carrier, adenine nucleotide translocator (*SLC25A4*) (Figure 3). Table 2 summarizes each of these 11 differentially expressed mitochondria-related genes and their functions. There were 25 genes in the array showing at least 1.5-fold changes in expression ($P = .001-.05$) at different time points (days 14, 21, 42, and 72). After Bonferroni adjustment for multiple comparisons only, *SLC25A23* was significantly down-regulated at all postradiation time points ($P = .008-.02$), and there was a significant change in expression in *FIS1*, *AIFM2*, *BCL2*, and *SLC25A4* in at least 1 postradiation time point.

Eight of the 11 differentially expressed genes were significantly associated with fatigue scores (*AIFM2*, *BCL-2*, *FIS1*, *IMMP2L*, *MSTO1*, *SLC25A23*, *SLC25A37*, and *SLC25A4*). Table 3 indicates the association between fatigue and mitochondria-related genes. Genes ($n = 25$) with more than a 1.5-fold change in expression at $P < .05$ at day 14, 21, 42, or 72 were subjected to pathway analysis using IPA. Functional networks of the 25 differentially expressed genes were examined to determine pathways that may explain the relationship between mitochondrial dysfunction and the development of CRF. The network analysis describes pathways that are related to cellular morphology, cellular assembly/organization, and cell death (Figure 4).

Discussion

To our knowledge, this is the first study to explore relationships between expression changes of genes related to mitochondrial integrity/function and self-reported fatigue in men with non-metastatic, localized prostate cancer receiving EBRT. Results from this hypothesis-generating study using an exploratory approach suggest an important role that mitochondria might play in the processes that create the sensation of fatigue in this population; whether this sensation is caused by inflammation, “central sensitization,” or other models have not

yet been well established. Although the expression of these mitochondria-related genes is triggered by multiple factors, it might serve as a signal that the body is finding a way to funnel multiple different stimuli into a common pathway to establish homeostasis. A recent study suggested that biochemical disturbances serve as the best predictors for the development of fatigue in women receiving radiotherapy for breast cancer.³¹ Fatigue could possibly be the body's signal, and the expressed mitochondria-related genes may be one of the common pathways signifying the body's attempt to establish homeostasis.

More than 90% of ATP is produced by mitochondrial oxidative phosphorylation through 2 coordinated metabolic processes—the tricarboxylic acid cycle and the electron transport/respiratory chain.³² The mitochondrial electron transport/respiratory chain is critical for maintaining effective ATP levels,³³ suggesting that a contributor to fatigue could be caused by a reduction in the capacity of mitochondria to use oxygen and synthesize ATP.^{34,35} It has been hypothesized that the inability of mitochondria to produce a sufficient supply of energy in the form of ATP plays a major role in fatigue.³⁵ Our results support this hypothesis by demonstrating that 11 genes associated with mitochondrial integrity and functions critical to ATP production were differentially expressed during EBRT. Eight of these 11 differentially expressed genes are directly involved in mitochondrial apoptosis pathway and signaling (*AIFM2*, *BCL2*, *BCL2L1*), mitochondrial membrane polarization and potential (*BCL2*, *BCL2L1*), mitochondrial transport (*BCL2*, *BCL2L1*, *IMMP2L*, *MIPEP*), and small molecular transport (*SLC25A23*, *SLC25A37*, *SLC25A4*).

The solute carrier family 25 (SLC25) consists of proteins that are code for mitochondrial transporters. The SLC25 family proteins transport molecules (ATP/ADT, amino acids, malate, ornithine, citrulline) from macromolecules to mitochondria to be converted into energy through oxidative phosphorylation.³⁶ Three differentially expressed genes (*SLC25A4*, *SLC25A23*, *SLC25A37*) found in this study are linked with SLC25 family proteins that not only trigger cellular injuries but also speed cellular death through disturbance in energy supply. For example, *SLC25A4* encodes the ADP/ATP translocator or adenine nucleotide translocator, which is the most abundant mitochondrial protein. The adenine nucleotide translocator determines the rate of ADP/ATP flux between the mitochondrion and the cytosol and regulates oxidative energy metabolism in cells. Adenine nucleotide translocator dysfunction (up- or down-regulated) is related to the pathogenesis of metabolic syndromes.³⁷ Second, mitoferrin-1 (Mfrn 1; *SCL25A37*) is located in the mitochondrial inner member and functions as an essential iron importer for the synthesis of mitochondrial heme and iron-sulfur cluster in erythroblasts.³⁸ Lastly, *SCL25A23* is a novel member of the mitochondrial solute carrier proteins, which encode the human isoforms of the ATP-Mg/Pi carrier in mitochondria and mediate the transport of metabolites across the inner mitochondrial membrane.³⁹ This is the first report to our knowledge that explores changes in gene expression of *SCL25A37* or *SCL25A23* in a human model. Our findings suggest that overexpression of *SCL25A37* and down-regulation of *SCL25A23* and *SLC25A4* are associated with fatigue symptoms because heme transport and oxidative energy metabolism are impaired in this population.

Radiation-induced free radicals (O_2^- , OH^- , ONO_2^-) frequently cause oxidative damage including the accumulation of defective proteins, increased mutation rates of mitochondrial

DNA, impairment of mitochondrial metabolism, and initiation of the mitochondrial/intrinsic pathway of apoptosis.^{18,40} The intrinsic apoptosis pathway is often activated in response to cell stress or damage such as those caused by radiation. It is regulated by the interaction of bcl-2 family members in the mitochondria.⁴¹ Activation of proapoptotic bcl-2 family members during intrinsic cell death leads to the formation of pores in mitochondrial outer membranes, followed by release of cytochrome C and other proapoptotic factors from the mitochondrial intermembrane space into the cytosol,⁴² and then triggers a conformational change to permit the apoptosome of caspase activation.⁴³

Our data confirmed the differential expression of genes linked with cell death as evidenced by the up-regulation of *BCL2L1* and down-regulation of *BCL2* and *AIFM2*, which are responsible for maintaining mitochondrial membrane integrity and resisting apoptosis. Differential expression of these genes (*BCL2L1*, *BCL2*, *AIFM2*) causes an imbalance in the interaction among bcl-2 proteins, leading to failure to inhibit *BAX* oligomerization. Impairing this cascade may lead to increased mitochondrial outer membrane permeability and consequently impair ability to release cytochrome C to resist apoptosis.⁴⁴ Our findings suggest that down-regulation of *BCL2* and *AIFM2* is associated with self-reported fatigue experienced by non-metastatic prostate cancer patients receiving EBRT. Follow-up studies investigating associations between expression of these mitochondria-related genes and levels of proinflammatory cytokines and/or markers of oxidative stress are warranted in order to investigate the role of these genes in the etiology of CRF or their utility to direct selective therapies for the efficient management of CRF. The association between the expression of the genes identified in this study and the variability in subjects' fatigue scores over time during EBRT also warrants further investigation to identify specific phenotypes of patients who are prone to develop fatigue during cancer treatment.

Limitations

In our preliminary findings, from this exploratory study using a small sample size, we were not able to demonstrate functional correlates between these genes and fatigue. The human mitochondria PCR array with 84 genes does not specifically target genes in the mitochondrial energy metabolism pathway that has been hypothesized to play a major role in the development of fatigue. However, this study contributes to knowledge related to signals for possible mechanisms of CRF through identification of 11 differentially expressed mitochondria-related genes in which 8 of these 11 genes showed significant associations with changes in mean fatigue scores reported by non-metastatic prostate cancer patients over time during EBRT. Further investigation is warranted to fully understand the relationship between gene expression and variations in fatigue scores of individual subjects during cancer treatment.

Conclusion and Implications

This study provides beginning empirical evidence that genes related to mitochondria and their function were differently expressed during EBRT and were associated with changes in fatigue symptoms reported by patients with nonmetastatic prostate cancer during EBRT. Our results warrant further research focused on 3 main areas: (a) exploration and validation of

the reported differentially expressed genes related to mitochondria, their functions, and their relationships with variations of fatigue scores reported by prostate cancer patients receiving EBRT; (b) confirmation of the relationship of the expressed mitochondria-related genes with other covariates to include but not be limited to age, race, disease severity, depression, symptom clusters; and (c) exploration of the role of other mitochondrial functions such as cellular energy expenditure or cellular apoptosis in the experience of CRF.

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Figure 1.

Piper Fatigue Score. Fifteen patients reported fatigue from prior to external beam radiation therapy (EBRT) (day 0), following EBRT (days 1 to 42) and 30 days after the last EBRT (day 72). One patient (patient 10) did not report any fatigue at any time point; 2 patients (patients 5 and 11) reported marked, sustained increases in fatigue following EBRT.

Figure 2.
Three up-regulated genes.

Figure 3.
Eight down-regulated genes.

Figure 4. The network pathways of combined observations from 4 time points (days 14, 21, 42, and 72). The threshold for analysis is 1.5-fold up- or down-regulation and $P < .05$. Copyright permission was obtained from Ingenuity Systems on February 18, 2011.

Table 1
Description of Sample Demographics and Clinical Characteristics

	Subjects (n = 15)				Controls (n = 15)				Reference Range
	Mean	SD	Range	n (%)	Mean	SD	Range	n (%)	
Age, y	62.8	8.6	49–81	15 (100)	57.2	7.6	45–76	15 (100)	
Ethnic									
White				10 (67)				10 (67)	
African American				3 (20)				3 (20)	
Others				2 (13)				2 (13)	
Clinical T stage									
T1 (a–c)				2 (13)					
T2 (a–c)				9 (60)					
T3 (a–c)				4 (27)					
Gleason score (median)	8	1	6–9						
Karnofsky score	89.3	2.6	80–90						
BMI	30.4	5.2	22.9–40.7						
Depression									
Baseline	1.5	2.6	0–8						
Completion	2.3	2.6	0–8						
PSA levels, ng/mL									0.0–4.0
Baseline	23.2	27.5	0.61–111						
Completion	0.04	0.01	0.04–0.05						
Hematocrit, %									40.1–51.0
Baseline	40.3	3.8	32.9–46.9						
Completion	36.8	2.9	33.0–42.0						
Albumin levels, g/dL									3.7–4.7
Baseline	4.1	0.3	3.5–4.5						
Testosterone, ng/dL									181–758
Baseline	243.9	159.9	20–505						
TSH, μ IU/mL									0.4–4.0
Baseline	1.8	1.2	0.17–3.8						
Total dosage of EBRT, Gy									
75.6				13 (87)					
68.4				2 (13)					

Abbreviations: BMI, body mass index; EBRT, external beam radiation therapy; PSA, prostate-specific antigen; TSH, thyroid-stimulating hormone.

Table 2
Expression Changes of 11 Mitochondria-Related Genes and Their Functions

Symbol	(↑/↓)	Fold Change/ <i>P</i> ^a	Full Gene Name	Function
<i>BCL2L1</i>	(↑)	1.33–3.17/.0007–.0421	BCL2-like 1	Antiapoptosis or proapoptosis; potent inhibitor of cell death
<i>FIS1</i>	(↑)	1.13–2.07/.0002–.0481	Fission 1 (mitochondrial outer membrane) homolog (<i>Saccharomyces cerevisiae</i>)	Regulates the morphology of mitochondria via balancing between fission and fusion in mitochondria
<i>SLC25A37</i>	(↑)	1.09–2.39/.0012–.0197	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 37	Localized in the mitochondrial inner membrane; an essential iron importer for the synthesis of mitochondrial heme and iron-sulfur clusters
<i>AIFM2</i>	(↓)	0.86–0.49/.0002–.0030	The apoptosis-inducing factor mitochondrion associated 2	Oxidoreductase, mediating a TP53/p53-dependent apoptosis response; a caspase-independent mitochondrial effector of apoptotic cell death
<i>BCL2</i>	(↓)	1.03–0.42/.0005–.0222	B-cell CLL/lymphoma 2	Proto-oncogene; suppresses apoptosis in a variety of cell systems
<i>IMMP2L</i>	(↓)	0.89–0.43/.0001–.0372	IMMP2L, IMP2	Catalyzes the removal of transit peptides required for the targeting of proteins from the mitochondrial matrix, across the inner membrane, into the inter membrane space
<i>MIPEP</i>	(↓)	0.99–0.50/.0065–.0233	Mitochondrial intermediate peptidase	Cleaves proteins, imported into the mitochondrion, to their mature size
<i>MSTO1</i>	(↓)	0.92–0.46/.0025–.0299	Misato homolog 1 (<i>Drosophila</i>)	Localized to the mitochondrial outer membrane; has a role in mitochondrial fission, distribution, and morphology
<i>NEFL</i>	(↓)	1.16–0.43/.0458–.0702	Neurofilament, light polypeptide	Neurofilaments are involved in the maintenance of neuronal caliber
<i>SLC25A23</i>	(↓)	0.94–0.32/.0001–.0002	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 23	Calcium-dependent mitochondrial solute carrier
<i>SLC25A4</i>	(↓)	0.95–0.43/.0003–.0131	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 4	Catalyzes the exchange of ADP and ATP across the mitochondrial inner membrane

Complete abbreviations of genes can be found at <http://www.sabiosciences.com>.

^aRange of fold-changes and *P* values for comparison of gene expression at each of 7 postradiation time points with baseline. Gene expressions related to mitochondria were evaluated using real-time PCR via the RT² ProfileR PCR Array System for 15 patients at baseline and 6 postradiation time points. The table lists the genes with greater than 2 fold up- or down-regulation changes or *P* < .05 for at least 1 postradiation time point compared with baseline.

Table 3
Association Between Fold Changes of Mitochondria-Related Genes and Fatigue Scores
Using Mixed Linear Effects Model

Mitochondrial Related Gene	Fatigue Score	
	β	Nonadjusted <i>P</i>
<i>AIFM2</i> ^a	-1.26	.006
<i>BCL2</i> ^a	-1.30	.0002
<i>BCL2L1</i>	.10	.22
<i>FIS1</i> ^a	.51	.02
<i>IMMP2L</i> ^a	-1.56	.0002
<i>MIPEP</i>	-.73	.06
<i>MSTO1</i> ^a	-1.71	.0003
<i>NEFL</i>	-2.22	.21
<i>SLC25A23</i> ^a	-1.08	.002
<i>SCL25A37</i> ^a	.43	.012
<i>SLC25A4</i> ^a	-.77	.028

^a Gene expression change is significantly associated with fatigue score.