Genomic Profile of Fatigued Men Receiving Localized Radiation Therapy

Chao-Pin Hsiao, PhD, RN^{1,2}, Swarnalatha Y. Reddy, PhD¹,
Mei-Kuang Chen, PhD³, and Leorey N. Saligan, PhD, RN, CRNP, FAAN¹

Abstract

Purpose: The purpose of this study was to explore gene expression changes in fatigued men with nonmetastatic prostate cancer receiving localized external beam radiation therapy (EBRT). **Methods:** Fatigue was measured in 40 men with prostate cancer (20 receiving EBRT and 20 controls on active surveillance) using the Functional Assessment of Cancer Therapy–Fatigue (FACT-F). EBRT subjects were followed from baseline to midpoint and end point of EBRT, while controls were seen at one time point. EBRT subjects were categorized into high- and low-fatigue groups based on change in FACT-F scores from baseline to EBRT completion. Full genome microarray was performed from peripheral leukocyte RNA to determine gene expression changes related to fatigue phenotypes. Real-time polymerase chain reaction and enzyme-linked immunosorbent assay confirmed the most differentially expressed gene in the microarray experiment. **Results:** At baseline, mean FACT-F scores were not different between EBRT subjects (44.3 ± 7.16) and controls (46.7 ± 4.32 , $p = .24$). Fatigue scores of EBRT subjects decreased at treatment midpoint (38.6 ± 9.17 , $p = .01$) and completion (37.6 ± 9.9 , $p = .06$), indicating worsening fatigue. Differential expression of 42 genes was observed between fatigue groups when EBRT time points were controlled. Membrane-spanning four domains, subfamily A, member (*MS4A1*) was the most differentially expressed gene and was associated with fatigue at treatment end point ($r = -.46$, $p = .04$). **Conclusion:** Fatigue intensification was associated with *MS4A1* downregulation, suggesting that fatigue during EBRT may be related to impairment in B-cell immune response. The 42 differentially expressed fatigue-related genes are associated with glutathione biosynthesis, γ -glutamyl cycle, and antigen presentation pathways.

Keywords

microarray, gene expression, fatigue, radiation therapy, prostate cancer, membrane-spanning four domains, subfamily A, member (*MS4A1*)

Prostate cancer is the second most common malignancy and the second leading cause of cancer mortality among American men (American Cancer Society, 2014). Localized external beam radiation therapy (EBRT) is a standard treatment option for men with nonmetastatic prostate cancer (Pinkawa & Gontero, 2009). Although EBRT has increased survival rates for men with prostate cancer, it is associated with numerous side effects, including distressing fatigue, not only during treatment (Truong et al., 2006) but also in disease-free states (Langston, Armes, Levy, Tidey, & Ream, 2013). Researchers have reported that fatigue intensifies at the midpoint of EBRT and declines after completion of the treatment (Miaskowski et al., 2008).

Fatigue is one of the most common and debilitating symptoms experienced by patients with cancer (X. S. Wang, 2008). Of all disease- and treatment-related symptoms in cancer, fatigue is among the most burdensome, with the greatest adverse effect on quality of life. Arguably, it is also among the least understood (Blackhall, Petroni, Shu, Baum, & Farace, 2009; Langston et al., 2013). The National Comprehensive

Cancer Network (NCCN, 2013) defines cancer-related fatigue (CRF) as a distressing, persistent sense of tiredness or exhaustion related to cancer or cancer treatment. CRF is associated with depression, impaired cognitive function, sleep disturbance, and decreased health-related quality of life (Berger & Mitchell, 2008; Byar, Berger, Bakken, & Cetak, 2006; Monga, Kerrigan, Thornby, & Monga, 1999). Although investigators have proposed many mechanisms to explain CRF

¹ National Institute of Nursing Research, Division of Intramural Research, National Institutes of Health, Bethesda, MD, USA

² The Frances Payne Bolton School of Nursing, Case Western Reserve University, Cleveland, OH, USA

³ Department of Psychology, University of Arizona, Tucson, AZ, USA

Corresponding Author:

Leorey N. Saligan, PhD, RN, CRNP, FAAN, National Institute of Nursing Research, National Institutes of Health, 3 Center Drive, Building 3, Room 5E14, Bethesda, MD 20892, USA.

Email: saliganl@mail.nih.gov

(Monga et al., 1997; Ryan et al., 2007; Saligan et al., 2013; St. Pierre, Kasper, & Lindsey, 1992; Yavuzsen et al., 2009), the cause remains elusive.

There is no optimal pharmacologic therapy for fatigue. The NCCN Practice Guidelines in Oncology for CRF currently recommend five nonpharmacological interventions: activity enhancement, psychosocial improvement, attention-restoring therapy, nutrition, and sleep (NCCN, 2013). For pharmacologic interventions, the NCCN guidelines recommend that, after ruling out other causes of fatigue, clinicians should consider the use of psychostimulants. One of the psychostimulants recommended is methylphenidate, which showed conflicting results in improving fatigue in two small randomized clinical trials (Bruera et al., 2006; NCCN, 2013). With limited options available to address CRF, novel strategies are needed to identify effective interventions.

Identification of clinical biomarkers and related functional pathways associated with CRF is a critical step to guide the development of effective therapies for the management of CRF (Menard et al., 2006). Our group has observed significant association between the upregulation of alpha-synuclein, a mediator of inflammation and neuroprotective pathways, and fatigue intensification during EBRT (Saligan et al., 2013). We also observed a significant relationship between radiation-related fatigue and the differential expression of genes related to mitochondrial biogenesis and bioenergetics (Hsiao, Wang, Kaushal, Chen, & Saligan, 2014; Hsiao, Wang, Kaushal, & Saligan, 2012). Other researchers have reported other biological pathways, candidate genes, and genetic markers for CRF, such as genes related to dopaminergic synapse (*COMT*, *DRD4*, and *DAT1*; Rausch et al., 2010) and cytokine–cytokine receptor interaction (interleukin [*IL*]-1 β , *IL*-1 receptor antagonist (*IL*-1RA), *IL*-6, and tumor necrosis factor [*TNF*]- α ; Aouizerat et al., 2009; Saligan & Kim, 2012; Thornton, Andersen, & Blakely, 2010). However, there are very limited association studies that propose genetic markers for CRF using a whole-genome approach (Sprangers et al., 2014). In the present study, we use whole-genome microarray to explore the association of changes in fatigue with changes in gene expression from RNA of peripheral blood mononuclear cells from fatigued men receiving EBRT for nonmetastatic prostate cancer.

Method and Material

The institutional review board of the National Institutes of Health (NIH), Bethesda, MD, approved the study (NCT00852111). We enrolled patients with nonmetastatic prostate cancer scheduled to receive localized EBRT (EBRT group) and those not treated but placed on active surveillance (control group). We excluded potential subjects from the study if they had progressive disease causing significant fatigue; had experienced major psychiatric illness within 5 years; had uncorrected hypothyroidism or anemia; took sedatives, steroids, or nonsteroidal anti-inflammatory agents; or had a second malignancy. After obtaining informed consent, we obtained demographic information and medical history by patient interview and review of medical records.

This study had a prospective, matched, case-controlled, repeated measures design. We enrolled participants from May 2009 to January 2012. We obtained peripheral blood samples and questionnaires from EBRT participants at baseline (prior to EBRT), midpoint (Days 19–21), and end point of the treatment (Days 38–42). To determine whether gene expression and fatigue score could be related to the stage of cancer or age, we recruited age- and race-matched men with nonmetastatic prostate cancer who were not receiving any form of treatment as controls for comparison of baseline data, gathering data from these participants at one time point only.

Clinical Measures

Fatigue. The Functional Assessment of Cancer Therapy–Fatigue (FACT-F) is a 13-item instrument measuring fatigue, with scores that range from 0 to 4 for each item (0 = *worst*, 4 = *best*) and 52 as the maximum possible score. The higher the FACT-F score, the lower the fatigue intensity (Yellen, Cella, Webster, Blendowski, & Kaplan, 1997). FACT-F has good reliability, with a .87 test–retest and internal consistency reliability with Cronbach's α .93, and is used extensively in individuals with cancer. Because research has shown that a 3-point change in FACT-F score is clinically important (Yost, Eton, Garcia, & Cella, 2011), we categorized the EBRT participants into large change in fatigue (high fatigue) and small change in fatigue (low fatigue) groups based on whether they demonstrated a change in FACT-F scores from baseline to the completion of EBRT ≥ 3 or < 3 , respectively.

Depression. We screened participants for depressive symptoms using the Hamilton Depression Rating Scale (HAM-D) at each time point. HAM-D is a 21-item, clinician-rated paper questionnaire with good internal reliability ($\alpha = .81-.98$). The predefined cutoff score for depression is 15 in cancer patients, with higher scores indicating more symptoms of depression (Lydiatt, Denman, McNeilly, Puumula, & Burke, 2008).

Microarray Chip Processing

We collected whole blood samples (2.5 ml) using PAXgene™ blood RNA (Qiagen, Frederick, MD) from each research subject at each time point. The PAXgene tubes were inverted 10 times to ensure red blood cell lysis immediately after blood collection and stored at -80°C until RNA extraction. RNA extractions and purification, amplification, hybridization, and scanning for chip array were performed by a single laboratory technician following a standard protocol to minimize nonbiological technical bias.

A total of 60 HG U133 Plus 2.0 affymetrix (Santa Clara, CA) chips were used to conduct microarray for RNA extracted from whole blood collected at baseline, midpoint, and end point of EBRT. The normalization of raw signal intensity values, transformation of quantile normalization value, detection of outliers, and statistical methods were performed according

to the procedures prescribed previously (Saligan et al., 2013; X. M. Wang et al., 2007).

Quantitative Real-Time Polymerase Chain Reaction (qPCR)

To confirm the most differentially expressed gene, we used RNA (120 ng) extracted from the same samples in the microarray experiment and converted to first-strand complementary DNA using the RT² First Strand kit (Qiagen; Hsiao, Araneta, Wang, & Saligan, 2013). Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and β -actin (*ACTB*) were used as reference genes. Primers for *GAPDH*, *ACTB*, and the most differentially expressed gene were used for normalization and amplification of data (Qiagen). When calculating delta cycle time (Ct) values, we used geometric means of Ct values of the two reference genes (Hsiao et al., 2013). All samples were tested in triplicate.

Enzyme-Linked Immunosorbent Assay (ELISA)

Expression of protein encoded by the most differentially expressed gene was confirmed by ELISA (, catalog # MBS-704400; MyBioSource, Inc., San Diego, CA) using cell pellets. The cell pellets were thawed on ice and lysed in two volumes of cell extraction buffer following the previous protocol (Hsiao et al., 2013). Protein concentrations were used for the normalization of cell number and determined using Pierce[®] bicinchoninic acid (BCA) protein assay kit (Thermo Scientific, Rockford, IL). ELISA was performed using 100 μ l of diluted cell pellets, following manufacturer's instruction, and tested in triplicate. The final concentration of each sample was normalized to the amount of cell lysate (milligram). The plates were read in a microplate reader VICTOR³ at 450 nm for 0.1 s.

Data Analysis

All statistical analyses were conducted using SPSS 19.0 (IBM Corporation, Armonk, NY) and R 3.0.0 for Windows.

Analysis related to clinical measures. Descriptive statistics were calculated for the participants' demographic and clinical characteristics. Repeated measures analysis of variance (ANOVA) was used to compare the mean fatigue score and gene expressions and the mean fatigue score and protein concentrations of the EBRT patients at baseline, midpoint, and end of the treatment. Individual growth curve analysis was used to describe how variables changed over time during EBRT from baseline to the end of the treatment.

Mixed linear model was used to estimate the individual growth curve of fatigue scores and gene/protein expression. We employed a linear individual change model in the analysis, that is, we assumed that the fatigue scores and the gene/protein expressions changed in a linear fashion over time. The intercept represents the initial status of the individual's fatigue level or gene/protein expression, and the slope indicates the rate of

change in the corresponding variables for each participant. To determine the associations between the changes in fatigue level and gene expression/protein concentration, the intercepts and slopes of fatigue scores and gene/protein expressions were estimated, respectively, and then correlated with each other.

Analysis related to microarray gene expression. Gene expression data by microarray were analyzed using Partek Genomics Suite software (Partek Inc., St. Louis, MO). The probe-level robust multichip average background correction, quantile normalization, and Log₂ transformation followed by probe-set summarization were performed on gene expression intensity values. Sample histograms and principal components analysis were performed as part of quality control analyses. To explore differentially expressed genes associated with fatigue, we performed analysis considering only *fatigue* in the ANOVA model for differential gene expression; further, we built three fatigue models separately based on each EBRT time point. Batch effects were controlled in the analysis by including the scanned dates in the model. The restricted maximum likelihood method was employed to fit the fixed and random effects of the model. For pairwise comparisons, multiple test correction was applied on the *p* values of the genes by the false discovery rate (FDR) step-up method introduced by Benjamini and Hochberg (1995). Differentially expressed gene lists were generated by a filtering criteria of a twofold change in expression (in either direction) and an FDR ≤ 0.05 level. The microarray data used in this article are uploaded in the NIH National Center for Biotechnology Information, Gene Expression Omnibus.

To uncover biological pathways and networks of genes that were differentially expressed between high- and low-fatigue groups during EBRT, pathway analyses were conducted using interactive pathway analysis (Qiagen Systems, Inc., Redwood City, CA). The significance value of the pathways was calculated by Fisher's exact test, right tailed.

Results

We enrolled 40 men with nonmetastatic prostate cancer in this study (20 men who received EBRT and 20 age- and race-matched controls). The majority of the men (80%, $n = 32$ of 40) were Caucasian, with a mean age of 64.2 (± 6.8) years for the entire sample. None of the 40 participants reached the cut-off score for clinical depression (mean = 0.85 $\pm$ 1.3; Lazure, Lydiatt, Denman, & Burke, 2009). A large majority ($n = 18$ [90%]) of the 20 EBRT subjects received a total dose of 75.6 Gray of EBRT, while the rest received a lower total dose of 68.4 Gray because of prior prostatectomy. Table 1 describes the demographic and clinical characteristics of the sample.

Fatigue

The mean FACT-F score did not differ significantly between EBRT participants at baseline (44.3 $\pm$ 7.16) and controls (46.7 $\pm$ 4.32, $p = .24$). Compared to baseline, mean FACT-F score for EBRT participants worsened significantly at

Table 1. Demographic and Clinical Characteristics of Sample.

Variable	EBRT (<i>n</i> = 20) Mean $\pm$ SD or <i>n</i> (%)	Control (<i>n</i> = 20) Mean ($\pm$ SD) or <i>n</i> (%)	<i>p</i> Value
Age, years	65.6 $\pm$ 7.5	62.8 $\pm$ 6.1	.36
Race			
Caucasian	16 (80)	16 (80)	
African American	4 (20)	4 (20)	
T-stage			
T1c	4 (20)	15 (75)	
T2a	9 (45)	5 (25)	
T2b–T2c	4 (20)		
T3a–T3b	3 (15)		
Gleason score			
6–7	9 (45)	20 (100)	
8–10	11 (55)		
Karnofsky Performance Scale	90.0 $\pm$ 0	95.2 $\pm$ 1.1	.89
Testosterone (ng/dl)	245.7 $\pm$ 168.5	217.9 $\pm$ 169.5	.78
Thyroid-stimulating hormone (μ U/ml)	2.01 $\pm$ 1.1	1.81 $\pm$ 1.3	1.01
PSA (ng/ml)	15.8 $\pm$ 13.2	2.68 $\pm$ 1.9	.02
Albumin (g/dl)	3.9 $\pm$ 0.3	4.2 $\pm$ 0.6	.98
Hemoglobin (mg/dl)	13.7 $\pm$ 0.9	13.1 $\pm$ 0.7	.46
Hematocrit (%)	40.3 $\pm$ 3.8	42.1 $\pm$ 1.7	.22
Depression (HAM-D)	1.2 $\pm$ 2.1	0.5 $\pm$ 0.6	.11
Total EBRT dosage (Gray)			
75.6	18 (90)	n/a	
68.4	2 (10)	n/a	

Note. *N* = 40. Controls were men with nonmetastatic prostate cancer not receiving any treatment who were placed on active surveillance. EBRT = external beam radiation therapy; HAM-D = Hamilton Depression Scale; PSA = prostate specific antigen; SD = standard deviation; T-stage = tumor stage (Tumor Nodes Metastasis classification for tumor staging).

midpoint (38.6 ± 9.2 , $p = .01$) and trended toward significance at completion of EBRT (37.6 ± 9.9 , $p = .06$), indicating intensification of fatigue during EBRT (Figure 1).

There were 5 men in the high-fatigue and 15 men in the low-fatigue groups based on the change in FACT-F score from baseline to EBRT end point. There were no significant differences in clinical characteristics between the fatigue groups. Compared to baseline (43.5 ± 7.58), there were significant changes in mean FACT-F score from baseline to midpoint (38.9 ± 8.94 , $p = .008$) and end point of EBRT (35.2 ± 10.0 , $p = .005$) in the high-fatigue group. There were no significant changes in mean FACT-F scores at any time point in low-fatigue group when compared to the baseline scores. There was a significant difference in mean fatigue scores at the end point between high- and low-fatigue groups ($p = .05$).

Gene Expression Profiles by Microarray

We detected 42 differentially expressed genes between high- and low-fatigue groups when controlling for EBRT time point. The most differentially expressed gene associated with fatigue was the membrane-spanning four domains, subfamily A, member 1 (*MS4A1*), with a difference in expression of -2.24 ($p = .0012$) between high- and low-fatigue groups at EBRT end point.

Confirmation by qPCR and ELISA

Using qPCR, we found no difference in *MS4A1* expression between the control group and EBRT subjects at baseline ($p = .97$). Similarly, we found no significant difference in *MS4A1* protein expression between the control group and EBRT subjects at baseline ($p = .51$). Both the effect of time

Figure 1. Changes in FACT-F scores in prostate cancer patients (*n* = 20) with EBRT at baseline, midpoint, and end point. *p* Values for midpoint and end point are relative to baseline. EBRT = external beam radiation therapy; FACT-F = Functional Assessment of Cancer Therapy–Fatigue.

Figure 2. Membrane-spanning four domains, subfamily A, member 1 (*MS4A1*) expression with changes of quantitative real-time polymerase chain reaction (qPCR) (a) cycle time (Ct number, an approximation method) and (b) fold change from baseline to midpoint (Days 19–21) and end point (Days 38–42) of external beam radiation therapy (EBRT). Ct represents level of gene expression: The lower the Ct value, the higher the gene expression level. The Ct value of *MS4A1* increased over time indicating a decrease in expression of *MS4A1*. Fold change decreased over time, also representing downregulation of *MS4A1*. * $p = .001$ relative to baseline value.

on *MS4A1* expression from baseline to completion of EBRT per qPCR cycle number and fold change were statistically significant ($F = 97.5$, $p = .001$; $F = 220.1$, $p = .001$, respectively), suggesting that cumulative EBRT dose influences the change in *MS4A1* expression. There was a significant association between changes in FACT-F scores and changes in *MS4A1* expression via qPCR from baseline to the end point of EBRT ($r = -.46$, $p = .02$). However, we observed no significant association between changes in FACT-F scores and changes in *MS4A1* protein concentrations in any of the study time points (Figure 2).

Biological Pathway and Networks

Biological pathways associated with the 42 genes that were differentially expressed between high- and low-fatigue groups include glutathione biosynthesis, γ -glutamyl cycle, and antigen presentation pathways ($p < .05$). Functional networks associated with these genes are functions of organismal injury and abnormalities, cellular development, cell-to-cell signaling and interaction, infectious disease, cell morphology, and cancer (Figure 3).

Discussion

To our knowledge, this study is the first to describe the genomic profile of fatigued men who are receiving localized radiation therapy for prostate cancer. In addition, to gain new insights into the possible mechanisms of radiation-associated fatigue, we utilized the microarray technique as an unbiased, hypothesis-generating approach. Our three major findings

include (a) prostate cancer patients experienced intensification of fatigue beginning at the midpoint and persisting until completion of EBRT, (b) *MS4A1* was the most differentially expressed fatigue-associated gene, and (c) biological pathways generated from the 42 fatigue-associated genes were glutathione biosynthesis, γ -glutamyl cycle, and antigen presentation pathways.

The trajectory of fatigue that we reported in this study is consistent with the findings of other studies (Langston et al., 2013; Miaskowski et al., 2008) and our previous findings (Hsiao et al., 2014, 2012; Saligan et al., 2013). These findings suggest that biologic mechanisms related to the repeated stress imposed by daily doses of irradiation are likely involved in the worsening of fatigue symptoms in this population. Further, the specific trajectory of fatigue intensification suggests that the implementation of interventions for treatment-associated fatigue should start early in the treatment.

Our findings suggest a possible therapeutic opportunity because the most differentially expressed gene associated with fatigue, *MS4A1*, is an epidermal growth factor receptor that binds to cell or plasma membrane. The *MS4A1* gene encodes a B-lymphocyte surface molecule, belonging to the CD20 superfamily, that differentiates B cells into plasma cells, and displays expression patterns unique among hematopoietic cells (Macardle & Nicholson, 2002; Riley & Sliwkowski, 2000). Human *CD20/MS4A1* functions to promote effective and optimal B-cell immune response (Fluge et al., 2011). The manufactured anti-CD20 monoclonal antibodies (anti-CD20 mAb), such as rituximab, are used to treat lymphoma, leukemia, and some autoimmune disorders (Edwards et al., 2004). These anti-CD20 mAbs bind to CD20, causing

Figure 3. Functional networks of 42 genes that are differentially expressed between high- and low-fatigue groups. [Coloring is based on the expression values of the genes, downregulation in green. Genes with no coloring are added from Ingenuity knowledge database.] Direct and indirect relationships are shown by solid and dashed lines, respectively. The arrow indicates specific directionality of interactions. The fold change of the genes is provided.

cytosolic calcium to increase, resulting in a signaling cascade that ends with the apoptosis of B cells (Walshe et al., 2008). *MS4A1* is not plotted in the pathway analysis in Figure 3 possibly because of insufficient findings in the literature on its association with the study's differentially expressed genes or because it corresponds to several loci.

Rituximab has also been used to successfully treat chronic fatigue syndrome (CFS; Lorusso et al., 2009). Researchers found that CFS patients who received rituximab experienced long-term improvements in self-reported fatigue scores (Fluge et al., 2011). Rituximab works to rapidly decrease the number of B cells by detection of *CD20/MS4A1* cell surface markers and enhance the initiation of the apoptosis pathway. This rapid B-cell depletion followed by an improvement in fatigue levels in response to treatment with rituximab suggests that CFS pathogenesis may involve an autoimmune disease mechanism (Fluge et al., 2011; Lorusso et al., 2009). *MS4A1* is thought to be a mediator in CFS etiology and is considered a key player in its overall mechanism. Landmark-Hoyvik et al. (2009) compared the white blood cell counts and B-cell pathways from breast cancer survivors with chronic fatigue and those without fatigue. The chronic fatigue group expressed higher numbers of leukocytes, lymphocytes, and

neutrophils (mean = 1.72) compared to the nonfatigued group (mean = 1.49, $p = .04$), indicating an overactive immune system in the fatigued group. A gene-set enrichment analysis of these groups also identified several gene sets related to pathways involving plasma, B cells, and the downregulation of B-cell responses in the chronic fatigue group. Landmark-Hoyvik et al.'s (2009) study supports the notion that B-cell immune response may play a role in fatigue related to cancer therapy and in other fatiguing conditions, such as in CFS.

In addition to the association of fatigue and B-cell immune response, the 42 differentially expressed fatigue-related genes ($p < .05$) in the present study are associated with three pathways: glutathione biosynthesis, γ -glutamyl cycle, and antigen presentation pathways. Specifically, one gene that was downregulated (-2.21) in the high-fatigue group, glutamate-cysteine ligase, plays a role in glutathione biosynthesis and γ -glutamyl cycle pathways (Figure 3). Glutathione regulates the redox state of protein cysteinyl thiols (Dalton, Shertzer, & Puga, 1999). Previous work showed that an increase in free radical formation causes cellular oxidative damage, producing physical or emotional fatigue (Jiang et al., 2013). Glutathione also plays a key role in cellular signaling, antioxidant defenses, and the regulation of proinflammatory cytokines (i.e., TNF- α ,

IL-1 β , IL-6; Haddad, 2002; Morris et al., 2014). Another down-regulated gene, *HLA-DRB3* (−2.497), is associated with the antigen presentation pathway. Antigen presentation in the immune system involves the processing of antigen and is central to the development of innate and adaptive immunity.

MS4A1 has indirect interaction with genes associated with cellular movement and immune cell trafficking, such as the carbonic anhydrase I (*CAI*) and the α -synuclein (*SNCA*) genes, although it has no direct interaction with the three pathways mentioned above. In a previous study, we noted that *SNCA* was upregulated in fatigued men during EBRT (Saligan et al., 2013). *CAI* belongs to the zinc metalloenzyme family, and functions by catalyzing the reversible hydration of carbon dioxide and participates in a variety of biological processes such as maintenance of acid–base balance, bone resorption, and calcification (Sly & Hu, 1995). Additionally, our group previously reported that overexpression of *IFI27*, which mediates parainflammatory response, oxidative stress, and proapoptotic signals, was associated with the intensification of CRF (Hsiao et al., 2013). We also previously found that an overexpression of *SNCA* along with changes in neuroinflammatory mechanisms was associated with development of fatigue in men during localized radiation therapy (Saligan et al., 2013). These genes may contribute to the cascade of biologic pathways that influence the intensification of fatigue as triggered by radiation therapy.

Limitations

We conducted this study in a tertiary research setting, with a semiselective patient population; therefore, the results may not be generalizable. Our hypothesis generating finding confirms the association of differentially expressed genes and fatigue intensification during EBRT, but it does not prove causation. Another limitation to this study is the small sample size; a larger sample should be pursued to confirm the associations of specific genes with fatigue. Additionally, collecting data at one time point from the control group limited our ability to longitudinally compare the trajectory of fatigue symptoms and gene/protein expressions in prostate cancer patients without the influence of radiation therapy.

Conclusion

The study findings provide preliminary evidence that B-cell response and the biological pathways of glutathione biosynthesis, γ -glutamyl cycle, and antigen presentation may play a role in the intensification of fatigue during EBRT. Also, functional networks associated with cellular development (morphology), cellular movement (signaling and interaction), and immune cell trafficking may influence fatigue symptoms. Further investigation is warranted using a larger sample to confirm these potential fatigue-related biological pathways and genetic molecular biomarkers.

The integration of genetic molecular measures into the study of CRF may provide better predictions of risk factor and

identification of prognostic biomarkers in translational research. Gaining a better understanding of molecular changes associated with EBRT and fatigue will lay the foundation for identifying opportunity for intervention in EBRT-associated fatigue.

Acknowledgments

We appreciate the assistance of Drs. Hyung-Suk Kim, Hyunhwa Lee, Maria Araneta, and Dan Wang, especially for serving as resources during the microarray gene expression, qPCR, and ELISA experiments.

Author Contribution

C-PH contributed to conception, design, and acquisition; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy. SYR contributed to analysis and interpretation, drafted the manuscript, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. M-KC contributed to analysis and interpretation, drafted the manuscript, critically revised the manuscript, gave final approval, and agrees to be accountable for all aspects of work ensuring integrity and accuracy. LNS contributed to conception, design, acquisition, and interpretation; drafted the manuscript; critically revised the manuscript; gave final approval; and agrees to be accountable for all aspects of work ensuring integrity and accuracy.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study is fully supported by the Division of Intramural Research of the National Institute of Nursing Research of the National Institutes of Health, Bethesda, MD (Protocol # 09-NR-0088).

References

- American Cancer Society. (2014). *Cancer facts & figures 2014*. Atlanta, Georgia: Author. Retrieved from <http://www.cancer.org/research/cancerfactsstatistics/cancerfactsfigures2014/>
- Aouizerat, B. E., Dodd, M., Lee, K., West, C., Paul, S. M., Cooper, B. A., . . . Miasowski, C. (2009). Preliminary evidence of a genetic association between tumor necrosis factor alpha and the severity of sleep disturbance and morning fatigue. *Biological Research for Nursing, 11*, 27–41.
- Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: A practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society. Series B (Methodological), 57*, 289–300.
- Berger, A. M., & Mitchell, S. A. (2008). Modifying cancer-related fatigue by optimizing sleep quality. *Journal of the National Comprehensive Cancer Network, 6*, 3–13.
- Blackhall, L., Petroni, G., Shu, J., Baum, L., & Farace, E. (2009). A pilot study evaluating the safety and efficacy of modafinil for

- cancer-related fatigue. *Journal of Palliative Medicine*, 12, 433–439.
- Bruera, E., Valero, V., Driver, L., Shen, L., Willey, J., Zhang, T., & Palmer, J. L. (2006). Patient-controlled methylphenidate for cancer fatigue: A double-blind, randomized, placebo-controlled trial. *Journal of Clinical Oncology*, 24, 2073–2078.
- Byar, K. L., Berger, A. M., Bakken, S. L., & Cetak, M. A. (2006). Impact of adjuvant breast cancer chemotherapy on fatigue, other symptoms, and quality of life. *Oncology Nursing Forum*, 33, E18–E26.
- Dalton, T. P., Shertzer, H. G., & Puga, A. (1999). Regulation of gene expression by reactive oxygen. *Annual Review of Pharmacology and Toxicology*, 39, 67–101.
- Edwards, J. C., Szczepanski, L., Szechinski, J., Filipowicz-Sosnowska, A., Emery, P., Close, D. R., . . . Shaw, T. (2004). Efficacy of B-cell-targeted therapy with rituximab in patients with rheumatoid arthritis. *New England Journal of Medicine*, 350, 2572–2581.
- Fluge, O., Bruland, O., Risa, K., Storstein, A., Kristoffersen, E. K., Sapkota, D., . . . Mella, O. (2011). Benefit from B-lymphocyte depletion using the anti-CD20 antibody rituximab in chronic fatigue syndrome. A double-blind and placebo-controlled study. *PLoS One*, 6, e26358.
- Haddad, J. J. (2002). The involvement of L-gamma-glutamyl-L-cysteinyl-glycine (glutathione/GSH) in the mechanism of redox signaling mediating MAPK(p38)-dependent regulation of pro-inflammatory cytokine production. *Biochemical Pharmacology*, 63, 305–320.
- Hsiao, C. P., Araneta, M., Wang, X. M., & Saligan, L. N. (2013). The association of IFI27 expression and fatigue intensification during localized radiation therapy: Implication of a para-inflammatory bystander response. *International Journal of Molecular Sciences*, 14, 16943–16957.
- Hsiao, C. P., Wang, D., Kaushal, A., Chen, M. K., & Saligan, L. (2014). Differential expression of genes related to mitochondrial biogenesis and bioenergetics in fatigued prostate cancer men receiving external beam radiation therapy. *Journal of Pain and Symptom Management*, 48, 1080–1090.
- Hsiao, C. P., Wang, D., Kaushal, A., & Saligan, L. (2012). Mitochondria-related gene expression changes are associated with fatigue in patients with nonmetastatic prostate cancer receiving external beam radiation therapy. *Cancer Nursing*, 36, 189–197.
- Jiang, D. Q., Guo, Y., Xu, D. H., Huang, Y. S., Yuan, K., & Lv, Z. Q. (2013). Antioxidant and anti-fatigue effects of anthocyanins of mulberry juice purification (MJP) and mulberry marc purification (MMP) from different varieties mulberry fruit in China. *Food Chemical Toxicology*, 59, 1–7.
- Landmark-Hoyvik, H., Reinertsen, K. V., Loge, J. H., Fossa, S. D., Borresen-Dale, A. L., & Dumeaux, V. (2009). Alterations of gene expression in blood cells associated with chronic fatigue in breast cancer survivors. *Pharmacogenomics Journal*, 9, 333–340.
- Langston, B., Armes, J., Levy, A., Tidey, E., & Ream, E. (2013). The prevalence and severity of fatigue in men with prostate cancer: A systematic review of the literature. *Supportive Care in Cancer*, 21, 1761–1771.
- Lazure, K. E., Lydiatt, W. M., Denman, D., & Burke, W. J. (2009). Association between depression and survival or disease recurrence in patients with head and neck cancer enrolled in a depression prevention trial. *Head & Neck*, 31, 888–892.
- Lorusso, L., Mikhaylova, S. V., Capelli, E., Ferrari, D., Ngonga, G. K., & Ricevuti, G. (2009). Immunological aspects of chronic fatigue syndrome. *Autoimmunity Reviews*, 8, 287–291.
- Lydiatt, W. M., Denman, D., McNeilly, D. P., Puumula, S. E., & Burke, W. J. (2008). A randomized, placebo-controlled trial of citalopram for the prevention of major depression during treatment for head and neck cancer. *Archives of Otolaryngology—Head and Neck Surgery*, 134, 528–535.
- Macardle, P. J., & Nicholson, I. C. (2002). Cd20. *Journal of Biological Regulators and Homeostatic Agents*, 16, 136–138.
- Menard, C., Johann, D., Lowenthal, M., Muanza, T., Sproull, M., Ross, S., . . . Camphausen, K. (2006). Discovering clinical biomarkers of ionizing radiation exposure with serum proteomic analysis. *Cancer Research*, 66, 1844–1850.
- Miaskowski, C., Paul, S. M., Cooper, B. A., Lee, K., Dodd, M., West, C., . . . Wara, W. (2008). Trajectories of fatigue in men with prostate cancer before, during, and after radiation therapy. *Journal of Pain and Symptom Management*, 35, 632–643.
- Monga, U., Jaweed, M., Kerrigan, A. J., Lawhon, L., Johnson, J., Vallbona, C., & Monga, T. N. (1997). Neuromuscular fatigue in prostate cancer patients undergoing radiation therapy. *Archives of Physical Medicine and Rehabilitation*, 78, 961–966.
- Monga, U., Kerrigan, A. J., Thornby, J., & Monga, T. N. (1999). Prospective study of fatigue in localized prostate cancer patients undergoing radiotherapy. *Radiation Oncology Investigations*, 7, 178–185.
- Morris, G., Anderson, G., Dean, O., Berk, M., Galecki, P., Martin-Subero, M., & Maes, M. (2014). The Glutathione system: A new drug target in neuroimmune disorders. *Molecular Neurobiology*, 50, 1059–1084.
- National Comprehensive Cancer Network. (2013). Cancer-related fatigue. *NCCN Clinical Practice Guidelines in Oncology: Cancer-related fatigue*. Retrieved from http://www.nccn.org/professionals/physician_gls/f_guidelines.asp
- Pinkawa, M., & Gontero, P. (2009). The motion: Radiotherapy for prostate cancer preserves sexual function to a greater extent than nerve sparing radical prostatectomy. *European Urology*, 56, 212–214.
- Rausch, S. M., Clark, M. M., Patten, C., Liu, H., Felten, S., Li, Y., . . . Yang, P. (2010). Relationship between cytokine gene single nucleotide polymorphisms and symptom burden and quality of life in lung cancer survivors. *Cancer*, 116, 4103–4113.
- Riley, J. K., & Sliwkowski, M. X. (2000). CD20: A gene in search of a function. *Seminars in Oncology*, 27, 17–24.
- Ryan, J. L., Carroll, J. K., Ryan, E. P., Mustian, K. M., Fiscella, K., & Morrow, G. R. (2007). Mechanisms of cancer-related fatigue. *The Oncologist*, 12, 22–34.
- Saligan, L. N., Hsiao, C. P., Wang, D., Wang, X. M., St John, L., Kaushal, A., . . . Dionne, R. A. (2013). Upregulation of alpha-synuclein during localized radiation therapy signals the association of cancer-related fatigue with the activation of inflammatory and neuroprotective pathways. *Brain, Behavior, and Immunity*, 27, 63–70.

- Saligan, L. N., & Kim, H. S. (2012). A systematic review of the association between immunogenomic markers and cancer-related fatigue. *Brain, Behavior & Immunity*, 26, 830–848.
- Sly, W. S., & Hu, P. Y. (1995). Human carbonic anhydrases and carbonic anhydrase deficiencies. *Annual Review of Biochemistry*, 64, 375–401.
- Sprangers, M. G., Thong, M. Y., Bartels, M., Barsevick, A., Ordoñana, J., & Shi, Q., . . . GeneQoL Consortium. (2014). Biological pathways, candidate genes, and molecular markers associated with quality-of-life domains: An update. *Quality of Life Research*, 23, 1997–2013.
- St. Pierre, B. A., Kasper, C. E., & Lindsey, A. M. (1992). Fatigue mechanisms in patients with cancer: Effects of tumor necrosis factor and exercise on skeletal muscle. *Oncology Nursing Forum*, 19, 419–425.
- Thornton, L. M., Andersen, B. L., & Blakely, W. P. (2010). The pain, depression, and fatigue symptom cluster in advanced breast cancer: Covariation with the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system. *Health Psychology*, 29, 333–337.
- Truong, P. T., Berthelet, E., Lee, J. C., Petersen, R., Lim, J. T., Gaul, C. A., . . . Ludgate, C. M. (2006). Prospective evaluation of the prevalence and severity of fatigue in patients with prostate cancer undergoing radical external beam radiotherapy and neoadjuvant hormone therapy. *Canadian Journal of Urology*, 13, 3139–3146.
- Walshe, C. A., Beers, S. A., French, R. R., Chan, C. H., Johnson, P. W., Packham, G. K., . . . Cragg, M. S. (2008). Induction of cytosolic calcium flux by CD20 is dependent upon B cell antigen receptor signaling. *Journal of Biological Chemistry*, 283, 16971–16984.
- Wang, X. M., Wu, T. X., Hamza, M., Ramsay, E. S., Wahl, S. M., & Dionne, R. A. (2007). Rofecoxib modulates multiple gene expression pathways in a clinical model of acute inflammatory pain. *Pain*, 128, 136–147.
- Wang, X. S. (2008). Pathophysiology of cancer-related fatigue. *Clinical Journal of Oncological Nursing*, 12, 11–20.
- Yavuzsen, T., Davis, M. P., Ranganathan, V. K., Walsh, D., Siemionow, V., Kirkova, J., . . . Yue, G. H. (2009). Cancer-related fatigue: Central or peripheral? *Journal of Pain and Symptom Management*, 38, 587–596.
- Yellen, S., Cella, D., Webster, K., Blendowski, C., & Kaplan, E. (1997). Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. *Journal of Pain and Symptom Management*, 13, 63–74.
- Yost, K. J., Eton, D. T., Garcia, S. F., & Cella, D. (2011). Minimally important differences were estimated for six Patient-Reported Outcomes Measurement Information System-Cancer scales in advanced-stage cancer patients. *Journal of Clinical Epidemiology*, 64, 507–516.