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Gene Expression, and Fatigue in Puerto Rican Men during Radiotherapy for Prostate Cancer: an exploratory study

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Abstract

Purpose/Objectives—To examine the trajectory of fatigue experienced by 26 Puerto Rican (PR) men over the course of External Beam Radiation Therapy (EBRT) and to assess gene expression changes from baseline to midpoint of EBRT using microarray technology.

Design/Research Approach—Prospective exploratory and comparative design study

Setting—RT facility located in San Juan, PR

Sample/Participants—26 PR men with non-metastatic prostate cancer

Methods—Participants completed 2 paper forms: demographics and the Spanish version of the 13-item FACT–fatigue at baseline, midpoint, and end of EBRT. Whole-blood samples were collected at baseline and at midpoint of EBRT. Descriptive data was analyzed using t-test, Wilcoxon, and Friedman test for repeated measures. Gene expression data was analyzed using the *LIMMA* package in R; the functional network analysis was conducted using Ingenuity Pathway analysis.

Main Research Variable—Fatigue scores, gene expression

Findings—Subjects were of ages 52–81 with fatigue scores that remained unchanged during EBRT (baseline=42.38, *SD*=9.34; midpoint=42.11, *SD*=8.93, endpoint=43.04, *SD*=8.62). Three hundred seventy-three genes (130-up regulated and 243-down regulated) were differentially expressed from baseline to mid-point of EBRT (FDR<0.01). The top distinct canonical pathways

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of the differentially expressed probesets (p< 0.0001) were: “Phospholipase C Signaling,” “Role of NFAT in Regulation of the Immune Response,” and “Gαq Signaling.”

Conclusions—While fatigue did not worsen over the course of EBRT for this sample as a group, there was variability in fatigue across the sample. It is possible that the over expression of the *SESN3* gene, known to suppress oxidative damage, may have contributed to the attenuation of fatigue in this clinical population.

Keywords

cancer related fatigue; Puerto Ricans; prostate cancer; radiotherapy; gene expression

Background

Prostate cancer (PC) is the most frequently diagnosed cancer (38.3% in 2012) in Puerto Rico and the leading cause of cancer death among Puerto Rican men (1). External beam radiation therapy (EBRT) is a more popular option in the management of non-metastatic PC cancer in Puerto Rico. About half of newly diagnosed PC patients in Puerto Rico received EBRT during the period of 2008–2012 (1). However, EBRT is not without side effects.

Fatigue is one of the most distressing and commonly reported side effect of EBRT, with up to 71% of PC patients complaining of it during EBRT (2). This fatigue has been associated with alterations in employment, increased hospitalizations, non-compliance with treatment, and need for dose adjustment or interruption of treatments (3). Despite the high prevalence of cancer-related fatigue (CRF) and the associated negative outcomes, much remains to be investigated, such as why there is variability of fatigue responses to EBRT treatment (4,5).

There is no singular explanation as to why this variability in the symptom experience of CRF during RT exists. Some factors that have been proposed include patient’s characteristics, and or treatment characteristics (6). However, there is increasing recognition that variability in the symptom experience may be explained by biological causal pathways (7). Recently, Saligan’s group (5,8) found that changes in fatigue scores from non-Hispanic white men with PC were significantly associated with changes in expression of mitochondrial-related and inflammation-related genes during EBRT.

An important gap in the CRF literature is that no studies have examined the trajectory of fatigue, specifically among Hispanic or Puerto Rican men during EBRT. It is crucial that clinicians and researchers become skilled in assessing the trajectory of fatigue of Puerto Rican PC patients undergoing EBRT to properly respond to their symptoms and unmet needs (9,10). Not only is there a need for more culturally competent research in this area, but because the Hispanic oncology population does not always report symptoms, risking under-assessment/management (11,12), the need for this exploratory study was particularly necessary. Therefore, the purpose of this study was to examine the trajectory of fatigue experienced by 26 Puerto Rican (PR) men over the course of External Beam Radiation Therapy (EBRT) and to assess gene expression changes from baseline to midpoint of EBRT using microarray technology.

Methods

For this prospective study, fatigue was measured from 26 Puerto Rican men newly diagnosed with non-metastatic PC at three time points: baseline [prior to EBRT], midpoint [days 19–21], and end of EBRT [days 38–42]. To capture the initial inflammatory response of EBRT, which peaks at midpoint (day 21), whole-blood samples were collected at baseline and at midpoint of EBRT to explore the differential expression of genes.

Prior to beginning data collection, approval by the Human Subjects Committee of both the Midwestern academic medical center and University of Puerto Rico Medical Science Campus was granted. Recruitment and data collection took place at an ambulatory RT facility located in San Juan, PR from July to September 2014. The inclusion criteria were Puerto Rican males over 40 years of age with a clinical diagnosis of non-metastatic PC, and who were scheduled for EBRT. Eligible participants also needed to be able to read and write at the 6th grade level and to provide written informed consent. Exclusion criteria include the following: progressive or unstable disease of any body system causing clinically significant fatigue; systemic infections; documented history of major depression, bi-polar 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 pro-inflammatory cytokines profiles.

Measures

Participants completed the demographics (only at baseline) and the Spanish version of the 13-item Functional Assessment of Cancer-Therapy –fatigue (FACT-F), at baseline, midpoint and end of EBRT. The principal investigator (PI) then administered the Hamilton depression scale (HDRS) validated in Spanish speaking populations, to obtain depression scores, and reviewed each subject's medical chart for recording selected clinical information on the health form.

The FACT-F Spanish version developed by Cella et al. (13) specifically for cancer patients measured fatigue severity. The FACT-F statements about fatigue (e.g. "I feel weak all over") were rated by the patients according to the degree to which each statement was true during the preceding week, using a five-point response scale (0 = not at all to 4 = very much). Test-retest scores suggest that FACT-F can be used independently overtime, and has been rigorously tested for reliability and validity with Hispanic cancer patients (14). FACT-F scores range between 0 and 52, where low scores mean higher intensity of fatigue. Cronbach alphas of 0.93–0.95 have been reported (15). The 21-item HDRS is a clinician-rated paper questionnaire that has been validated in Spanish speaking population with good psychometric properties (i.e. Cronbachα>0.7, ICC: >0.9) (16).

A total of 2.5 mL of peripheral blood was collected using RNA PAXGene tubes (Qiagen Frederick, MD) for each of the two study time points. De-identified blood samples were stored in a securely- locked −80°C freezer until shipment to Dr. Saligan's laboratory at the National Institute of Nursing Research at NIH. RNA extraction, purification, cDNA and cRNA synthesis, amplification, hybridization, scanning and data analyses were conducted in the NINR lab following standard protocols described in a prior publication (5). Fifty-two

Affymetrix microarray chips (HG U133 Plus 2.0, Santa Clara, CA) were used to assess gene expression levels for genes across the genome. The AffymetrixGeneChip Command Console (AGCC, 3.0 V) was used to scan the images for data acquisition.

Statistical analysis

Power and sample size needed to detect differentially expressed genes between baseline and midpoint of EBRT was computed for 80% power and a standard deviation of 0.70 using the web application at http://bioinformatics.mdanderson.org/MicroarraySampleSize/. 26 subjects met the 80% power criterion to detect a minimum of 2.0 fold change in gene expression from baseline to midpoint of EBRT.

To assess the change in fatigue at the three time-points, we compared all pair-wise fatigue scores measured at the three time points using both parametric (paired t-tests) and non-parametric (Wilcoxon Signed Rank Test) tests to assess the robustness of results. In addition, we conducted subset analysis and case levels of the fatigue scores using descriptive statistics.

Affymetrix CEL files (the file format that stores the results of the intensity calculations of the pixel values of the scanned image from a microarray chip) were imported into *LIMMA* package in R. Background correction, quantile normalization, and summarization expression calculation were conducted with the Robust Multichip Average (RMA) algorithm and the linear model with *LIMMA* package. In addition to the laboratory quality control steps, after RMA, data quality was assessed via per-subject box plots (Figure 1). Gene expression differences between midpoint and baseline of EBRT were assessed using the linear modeling features available in the *LIMMA* package. To adjust for the testing of thousands of genes, we used the approach of Benjamini-Hochberg to estimate the false discovery rate (FDR). Genes with a FDR < 0.01 and a fold change >0.4 in either direction were considered top candidate differentially-expressed genes. Further, the 646 probeset with a $p < 0.0001$ from midpoint to baseline of EBRT were subjected to pathway analysis using Ingenuity Pathway (IPA) (Ingenuity® Systems, www.ingenuity.com, Redwood City, CA). The cutoff log ratio was set at 0.4 providing the opportunity to analyze only those highly up-regulated and down-regulated differentially expressed genes ($N = 220$ genes). IPA identified functional networks of the differentially expressed genes from the Ingenuity's Knowledge Base.

Results

Sample

Participants (N = 26) had a mean age of 67.01, with a *SD* of 7.56. Most of the participants were White (85%), and retired (69.2%) (Table 1). While 96.2% of the participants were married or partnered, three (11.5%) participants reported that they cared for themselves on their own or were caring for their dementia-affected spouse. The participants were well educated with only four (15.4%) not having a high school diploma.

Clinical Characteristics

Approximately one third of the sample (34.6%) had low risk disease with a PC clinical stage of T1 and a Gleason score between six and nine, 46.2% had stage T2, 15.4% had stage T3, and one participant had stage T4 prostate cancer (Table 2). Most participants (57.7%) received neo-adjuvant hormonal therapy (androgen deprivation therapy; ADT) eight weeks prior to the initiation of EBRT, and only 23.1% received radical prostatectomy more than a year before receiving EBRT. Baseline hemoglobin, albumin, and TSH were within reference range.

Trajectory of Fatigue

Mean fatigue score at baseline was 42.38 for the sample (Table 3). It remained about the same at mid-point 42.11, and slightly increased to 43.03 at completion of EBRT, but was not significantly statistically different. We also conducted the Friedman's test that is the nonparametric test equivalent to the repeated measures ANOVA. Those results also confirmed no significant difference (Friedman's test: $\chi^2$1.20 [*df*, 2], p =.55).

While as a group there were no *statistically* significant changes in fatigue over the course of EBRT, subset analysis of the fatigue scores showed that, of the 26 subjects, some of the participants had *clinically* significant change (i.e. > 3 point decrease) in fatigue (baseline to midpoint: 5 (20%); baseline to endpoint: 4 (15%); and, midpoint to endpoint: 3 (11.5%). None of the participants reached the cutoff score (15) for depression at any time point. Lastly, the FACT-F scale showed acceptable internal reliability. The range of the Cronbach alphas for the FACT-F scale across the three time points was .91–.93, and for the HDRS was .55–.84.

Global Gene expression analysis

Three hundred and seventy three genes (130 up-regulated and 243 down-regulated) were differentially expressed from baseline to midpoint of EBRT FDR <0.01. Figure 2 illustrates a volcano plot of results for all genes for determining differentially expressed genes from baseline to midpoint of EBRT. Further, the list of the top 20 significant up/down-regulated genes accounting for both statistical and biological significance is shown in table 4. Table 4 also shows that genes from this top list were also observed to be the top 20 up/down-regulated genes in a similar study among non-Hispanic white men.

Pathway Analyses

In order to obtain a better understanding of the physiologic pathways that are associated with the genes that are differentially expressed from baseline to midpoint of EBRT, a functional network analysis was conducted. A network of the top three canonical pathways (Phospholipase C Signaling, Role of NFAT in Regulation of the Immune Response, Gαq Signaling) and its associated differentially expressed genes was generated (see Figure 3).

Discussion

Trajectory of Fatigue

The purpose of this exploratory study was to describe the trajectory of fatigue among Puerto Rican men over the course of receiving EBRT for non-metastatic PC and to explore gene expression changes from baseline to midpoint of EBRT. We employed well-validated and reliable Spanish versions of instruments commonly used in other studies and that have been found sensitive to changes over time to assess fatigue and other symptoms. This sample was carefully assessed for potential confounding factors (e.g. renal failure, anemia) resulting in participants with relatively homogeneous characteristics. In the gene expression analyses, measures were taken to correct for false positive results that may arise from high-through output techniques.

Fatigue is a side effect of RT that can negatively impact their health related quality of life and physical functioning (17). At baseline and at end of treatment respectively, FACT-F mean scores of the sample was similar (42.38, $SD = 9.34$ and 43.03, $SD = 8.62$, respectively) compared to previously published mean fatigue score of the U.S. general population using the same measure (43.6, $SD = 9.4$) (18). At each of the three time point the Puerto Rican participants reported higher FACT-F mean scores, indicating less level of fatigue as compared to: Canadian PC men receiving ADT ($M = 37$; $SD = 24$) (19); US non-anemic ($M = 13.5$; $SD = 1.2$) (18) cancer patients; and US cancer patients receiving chemotherapy/radiotherapy ($M = 30.1$, $SD = 13.1$) (20) using the same measure of fatigue. Our smaller sample size may have contributed in part to the observed differences in fatigue, but it may also be due to the above studies dealing with more aggressive cancer types and/or more intense treatments.

Longitudinal studies have consistently reported that fatigue in PC patients worsens over the course of EBRT (5,8,21). The results of this study do not support these findings. Unexpectedly, there were no significant differences in fatigue scores over time during EBRT in our Puerto Rican sample. In comparison, the work by Saligan et al. (5,8) with a non-Hispanic white sample found that, compared to baseline, fatigue increased significantly at midpoint and at completion of EBRT ($p = .001–.04$) using the Piper Fatigue Score and the PROMIS-F. While similar EBRT protocols and data collection time points were used, differences in assessment scales also may affect the observed results (22). Although these instruments may have good psychometric properties, they may not lead to the same conclusions since they may also assess different aspects or characteristics of fatigue. For example, the Piper Fatigue Scale assesses four dimensions of fatigue: behavioral/severity, sensory, cognitive/mood, and affective (23). Nonetheless, since the FACT- F is unidimensional, it is possible that some other dimensions of fatigue not assessed in the present study might have changed over the course of EBRT. In addition to using different instruments to measure fatigue, these other studies had different sample sizes and were not limited to Hispanic participants. Due to these variations, direct comparisons between these studies and our study are limited.

Changes in Gene Expression

Radiation therapy induces damage to the cell DNA that results in a cascade of events involving a network of signal transduction and transcriptional regulation (24). This event stimulates a cellular stress response, including DNA damage recognition and cell cycle arrest that leads to either DNA repair or apoptosis. In our study, we used microarray technology to conduct a genome wide study focused on the identification of transcriptional changes resulting from the radiotherapy insult from baseline to midpoint of EBRT. The 20 most up- and down-regulated genes include significant changes in expression of genes related to apoptosis (*FDRX*), B-cell functions (*FCRLA, IGHM, PAX 5, BACH2, MS4A1,* and *POU2AF1),* inflammation *(ITLN1, CCR7*), hemoglobin synthesis (*XK and RHD*), and induced by oxidative stress (*SESN3*).

During EBRT, there is active cellular apoptosis (5). Consistent with this, the present study findings showed that apoptosis-related genes including ferredoxin reductase (*FDRX*) and sestrin 3 (*SESN3*) were up-regulated during EBRT. Ferredoxin reductase *FDXR* was the most up-regulated observed in this study. *FDXR* is regulated by the p53 family by DNA damaging agents in a p53-dependent manner, and by a mutated form of p53 that is involved in inducing apoptosis (25). A previous study demonstrated that over-expression of the ferredoxin reductase protein increased the sensitivity of colorectal carcinoma cells to reactive oxygen species (ROS) 5-FU, and doxorubicin-induced cell death (25). Specifically, the mechanism by which *FDXR* regulates ROS induced apoptosis is by hindering ROS from being detoxified by an antioxidant system (25). ROS production is a critical process linked to RT-related fatigue (5). It has been suggested that since ROS is mainly produced in the mitochondria, it may have an effect on oxidizing the mitochondrial pores resulting in disruption of the mitochondrial membrane potential that can consequently lead to cytochrome c release and apoptosis (5,25). The current study findings and the supporting evidence strengthens our hypothesis that *FDXR* up-regulation accelerates ROS-induced apoptosis, which may contribute to the experience of fatigue in this clinical population.

The current study also revealed that the *SESN3* gene was significantly up-regulated during EBRT. *SESN3* is known to suppress oxidative damage (26). Accumulation of ROS in the mitochondria serves as an apoptotic stimulus of the intrinsic death pathway (27). Extrinsic factors such as EBRT can lead to oxidative stress (28). Oxidative stress is the reflection of an imbalance of ROS and reactive nitrogen species (RNS) metabolism and inability of the cells (mitochondria) to detoxify ROS and RNS and other reactive metabolic intermediates (26). While all members of the sestrin family (*SESN1*, *SESN2*, and *SESN3*) are induced by oxidative stress by different induction mechanism, *SESN3*, which is highly up-regulated in the present study, is stimulated by oxidative damage via activation of FOXO transcription factors (27). FOXO3 also was up-regulated (*adjusted p value* <.02, log fold change 0.20) in this study. *SESN3* knockdown caused an increase of FOXO3 inducing ROS and accelerating apoptosis. SESN3, a FOX-inducible protein, has been shown to suppress oxidative stress-induced mTORCH 1 (target of rapamycin) activities, thus maintaining cellular energy during oxidative stress (26,27). Although it is not clear that FOXO3 activated *SESN3* in our study, it is plausible that the antioxidant, AMPK-activating, and MTORC1 suppressing capabilities of the sestrin family contributed to the attenuation of RT-related fatigue in this clinical

population. More recently, evidence suggest an important role of Sestrins in behavioral symptoms (e.g. fatigue, cognitive impairment, and pain) as well (29–31). Additional studies are needed to characterize the expression of *FDRX* and the Sestrin genes, and to determine their role in RT-related injury and fatigue or in other in behavioral symptoms.

Because the expression of 646 genes was found to be differentially expressed ($p < .00001$) between midpoint and baseline of EBRT, we further explored the canonical pathways in which these genes may be involved. The top three identified functional pathways--phospholipase c signaling, role of NFAT in regulation of the immune response, and Gαq signaling. These pathways have been demonstrated in previous research to be involved in immune regulation and the complex signaling pathway that regulates cell dynamics (32). However, since most of the genes from the top three pathways except *GNAS*, were down-regulated, this finding supports the idea that induction of RT in our sample may has been balanced by suppression of these genes based on the intensity of the damage. Nonetheless, these functional pathways suggested biological underpinnings of the possible physiological mechanisms that influence fatigue modulation during EBRT in this population.

One of the most highly ranked pathways was Role of NFAT in Regulation of the Immune, which is predominantly marked by the up-regulation of *GNAS* and the down-regulation of 8 genes (*IP3R, CSP, BCR, SLP65, LCK, FYN, CALM, P13K*). NFAT are a family of transcription factors expressed in several cell types of the immune system, therefore playing an important role in immune responses process (32). NFAT are activated by stimulation of receptors coupled to Calcium-Calcineurin signals from various kinases (32). For example, the current study findings showed that the ATM serine/threonine kinase (log fold change= −0.510, p = .000043) was significantly down-regulated, suggesting that the NFAT transcription factors are not activated during EBRT. However, it also has been suggested that NFATc signaling pathway plays a role in dysregulation of inflammation (33). The activation of the NFATc signaling pathway in macrophages leads to a hyperinflammatory effect with immune-pathologic consequences (33). The acute inflammation during RT has been associated with fatigue (34). Since NFATc isoforms actively and negatively are regulated in macrophages during acute inflammatory responses (33) suggesting that any deregulation leading to NFATc activation (e.g. RT) might lead to excessive pro-inflammatory cytokine production and to the development to RT-related fatigue. However, none of these cytokines (IL-1b, IL-6, TNF-a) were differentially expressed in our study ($p > .05$).

Another ranked pathway was the Gαq Signaling pathway. Similar to the previous pathways discussed above, *GNAS* was the only up-regulated gene (expression value 0.573); while the other genes associated with these pathways are down-regulated (*ATM, CAMK4, FNBPI, ITPR1, NAPEPLD, PIK3R1, PRKA, PRKCH,* and *RHOH*). After activation, the G-proteins route the signal molecules from cell surfaces receptors that are activated by ligands such as hormones, neurotransmitters and chemokines to regulate diverse physiological functions (32). Whistler et al. (35) identified 839 fatigue-associated genes among 112 female subjects with unexplained fatigue. Unexplained fatigue was measured with the Multidimensional Fatigue Inventory. The authors did not provide the entire list of genes associated with fatigue; however, they provided the results of mapping fatigue-associated genes to pathways. These associated genes implicated six different signaling pathways including the G-protein

signaling pathway. This similarity to the present study suggests that Gαq Signaling pathway might have played a role in fatigue development. Further studies are needed to confirm that these functional pathways are indeed involved in RT-related fatigue.

Limitations of this study include the relatively small sample size (despite our power analysis). A larger sample would have permitted a subset analysis of those participants for whom fatigue worsened, something we were unable to do due to the small sample size. Using a unidimensional measure of fatigue, also may have been a limitation. As fatigue is multi-dimensional, it is possible our measure did not capture that part of fatigue that might have increased over the course of EBRT for more study participants. The planned analysis of association between the changes in expression level of these genes with changes in fatigue scores from baseline to midpoint of EBRT was not conducted due to the unexpected finding that fatigue did not significantly change over the course of EBRT. Additional exploration of variables of interest (i.e. consumption of a high antioxidant diet), is required to confirm if these have contributed to the attenuation of RT-related fatigue in the current study clinical population.

Conclusion

EBRT for PC is a popular treatment option in Puerto Rico, and findings from this study suggest it may have a less detrimental effect on fatigue than in the non-Hispanic white population. While fatigue did not worsen over the course of EBRT for this sample as a group, there was variability in fatigue across the sample. Nonetheless, it is particularly important to understand the prevalence and the trajectory of fatigue during cancer treatments in order to develop and deliver timely targeted interventions in comprehensive oncology care (36). Further research using qualitative methodologies may be helpful to better understand why the Puerto Rican men in this study did not experienced changes in fatigue.

Although it is unclear from our study whether the changes in gene expression we found were specifically associated with fatigue, global differentially expressed genes during EBRT suggest activation of immune response, which is a mechanism proposed to explain cancer-related fatigue. Future research will be directed at providing direction toward the identified genes and pathways that may contribute to our understanding of the complexity of RT-related fatigue. This is particularly important for the PC population because of the potential disabling symptom during and after EBRT. Nonetheless, the contribution of gene expression studies in developing personalized cancer therapies seems promising. It will contribute to making use of individual differences in symptom experience to identify genetic and epigenetic risk factors for developing symptom variability.

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References

1. Population Source: US Census Bureau Inter-censal Estimates. Puerto Rico Health Department, Puerto Rico Central Cancer Registry; 2016. Puerto Rico Central Cancer Registry, Incidence/Mortality/Radiotherapy Case File 2008–2012.
2. Langston B, Armes J, Levy A, Tidey E, Ream E. The prevalence and severity of fatigue in men with prostate cancer: a systematic review of the literature. Support Care Cancer. 2013; 21:1761–71. [PubMed: 23455492]
3. Franklin DJ, Packel L. Cancer-related fatigue. Arch Phys Med Rehabil. 2006; 87:S91–3. quiz S4–5. [PubMed: 16500197]
4. Fransson P. Fatigue in prostate cancer patients treated with external beam radiotherapy: a prospective 5-year long-term patient-reported evaluation. J Cancer Res Ther. 2010; 6:516–20. [PubMed: 21358092]
5. Hsiao CP, Wang D, Kaushal A, Saligan L. Mitochondria-related gene expression changes are associated with fatigue in patients with nonmetastatic prostate cancer receiving external beam radiation therapy. Cancer Nurs. 2013; 36:189–97. [PubMed: 23047795]
6. Hickok JT, Roscoe JA, Morrow GR, Mustian K, Okunieff P, Bole CW. Frequency, severity, clinical course, and correlates of fatigue in 372 patients during 5 weeks of radiotherapy for cancer. Cancer. 2005; 104:1772–8. [PubMed: 16116608]
7. Reyes-Gibby CC, Spitz M, Wu X, Merriman K, Etzel C, Bruera E, et al. Cytokine genes and pain severity in lung cancer: exploring the influence of TNF-alpha-308 G/A IL6-174G/C and IL8-251T/A. Cancer Epidemiol Biomarkers Prev. 2007; 16:2745–51. [PubMed: 18086782]
8. Saligan LN, Hsiao CP, Wang D, Wang XM, St John L, Kaushal A, et al. Upregulation of alpha-synuclein during localized radiation therapy signals the association of cancer-related fatigue with the activation of inflammatory and neuroprotective pathways. Brain Behav Immun. 2013; 27:63–70. [PubMed: 23022913]
9. Gonzalez V, Tirado M, Hughes D, Pedro E, Velez M. Identification of supportive care needs in a sample of Puerto Rican cancer patients with the supportive care needs survey. Journal of Nursing Education and Practice. 2015; 5:32–38.
10. Gonzalez VJ, McMillan SC, Groer M, Imbert S, Pedro E, Tome J. Symptom Burden: Experiences of Puerto Rican Men With Prostate Cancer Prior to External Beam Radiation Therapy. Clin J Oncol Nurs. 2017; 21:104–12. [PubMed: 28107324]
11. Im EO, Chee W, Lim HJ, Liu WM. An online forum exploring needs for help of patients with cancer: gender and ethnic differences. Oncol Nurs Forum. 2008; 35:653–60. [PubMed: 18591169]
12. Jacob E, McCarthy KS, Sambuco G, Hockenberry M. Intensity, location, and quality of pain in Spanish-speaking children with cancer. Pediatr Nurs. 2008; 34:45–52. [PubMed: 18361086]
13. Yellen SB, Cella DF, Webster K, Blendowski C, Kaplan E. Measuring fatigue and other anemia-related symptoms with the Functional Assessment of Cancer Therapy (FACT) measurement system. J Pain Symptom Manage. 1997; 13:63–74. [PubMed: 9095563]
14. Baro E, Carulla J, Cassinello J, Colomer R, Mata JG, Gascon P, et al. Psychometric properties of the Perform Questionnaire: a brief scale for assessing patient perceptions of fatigue in cancer. Support Care Cancer. 2011; 19:657–66. [PubMed: 20432046]
15. Cella D, Eton DT, Lai JS, Peterman AH, Merkel DE. Combining anchor and distribution-based methods to derive minimal clinically important differences on the Functional Assessment of Cancer Therapy (FACT) anemia and fatigue scales. J Pain Symptom Manage. 2002; 24:547–61. [PubMed: 12551804]
16. Bobes J, Bulbena A, Luque A, Dal-Re R, Ballesteros J, Ibarra N. A comparative psychometric study of the Spanish versions with 6, 17, and 21 items of the Hamilton Depression Rating Scale. Med Clin (Barc). 2003; 120:693–700. [PubMed: 12781095]
17. Truong PT, Berthelet E, Lee JC, Petersen R, Lim JT, Gaul CA, et al. Prospective evaluation of the prevalence and severity of fatigue in patients with prostate cancer undergoing radical external

beam radiotherapy and neoadjuvant hormone therapy. The Canadian journal of urology. 2006; 13:3139–46. [PubMed: 16813705]

18. Cella D, Lai JS, Chang CH, Peterman A, Slavin M. Fatigue in cancer patients compared with fatigue in the general United States population. Cancer. 2002; 94:528–38. [PubMed: 11900238]
19. Richard PO, Fleshner NE, Bhatt JR, Hersey KM, Chahin R, Alibhai SM. Phase II, randomised, double-blind, placebo-controlled trial of methylphenidate for reduction of fatigue levels in patients with prostate cancer receiving LHRH-agonist therapy. BJU Int. 2015; 116:744–52. [PubMed: 24684534]
20. Henry DH, Viswanathan HN, Elkin EP, Traina S, Wade S, Cella D. Symptoms and treatment burden associated with cancer treatment: results from a cross-sectional national survey in the U.S. Support Care Cancer. 2008; 16:791–801. [PubMed: 18204940]
21. Hsiao CP, Araneta M, Wang XM, Saligan LN. The association of IFI27 expression and fatigue intensification during localized radiation therapy: implication of a para-inflammatory bystander response. Int J Mol Sci. 2013; 14:16943–57. [PubMed: 23959120]
22. Aaronson LS, Teel CS, Cassmeyer V, Neuberger GB, Pallikkathayil L, Pierce J, et al. Defining and measuring fatigue. Image J Nurs Sch. 1999; 31:45–50. [PubMed: 10081212]
23. Piper BF, Dibble SL, Dodd MJ, Weiss MC, Slaughter RE, Paul SM. The revised Piper Fatigue Scale: psychometric evaluation in women with breast cancer. Oncol Nurs Forum. 1998; 25:677–84. [PubMed: 9599351]
24. Jen KY, Cheung VG. Transcriptional response of lymphoblastoid cells to ionizing radiation. Genome Res. 2003; 13:2092–100. [PubMed: 12915489]
25. Liu G, Chen X. The ferredoxin reductase gene is regulated by the p53 family and sensitizes cells to oxidative stress-induced apoptosis. Oncogene. 2002; 21:7195–204. [PubMed: 12370809]
26. Lee JH, Budanov AV, Karin M. Sestrins orchestrate cellular metabolism to attenuate aging. Cell Metab. 2013; 18:792–801. [PubMed: 24055102]
27. Hagenbuchner J, Kuznetsov A, Hermann M, Hausott B, Obexer P, Ausserlechner MJ. FOXO3-induced reactive oxygen species are regulated by BCL2L11 (Bim) and SESN3. J Cell Sci. 2012; 125:1191–203. [PubMed: 22349704]
28. Gong B, Chen Q, Almasan A. Ionizing radiation stimulates mitochondrial gene expression and activity. Radiat Res. 1998; 150:505–12. [PubMed: 9806591]
29. Rai N, Kumar R, Desai GR, Venugopalan G, Shekhar S, Chatterjee P, et al. Relative Alterations in Blood-Based Levels of Sestrin in Alzheimer's Disease and Mild Cognitive Impairment Patients. J Alzheimers Dis. 2016; 54:1147–55. [PubMed: 27567861]
30. Kallenborn-Gerhardt W, Lu R, Syhr KM, Heidler J, von Melchner H, Geisslinger G, et al. Antioxidant activity of sestrin 2 controls neuropathic pain after peripheral nerve injury. Antioxid Redox Signal. 2013; 19:2013–23. [PubMed: 23495831]
31. Broderick G, Craddock RC, Whistler T, Taylor R, Klimas N, Unger ER. Identifying illness parameters in fatiguing syndromes using classical projection methods. Pharmacogenomics. 2006; 7:407–19. [PubMed: 16610951]
32. Ingenuity Pathway Analysis (IPA)(Ingenuity® Systems. Redwood City, CA: www.ingenuity.comAvailable at: URL: http://www.ingenuity.com/products/login [Accessed March 15, 2015]
33. Zanoni I, Granucci F. Regulation and dysregulation of innate immunity by NFAT signaling downstream of pattern recognition receptors (PRRs). Eur J Immunol. 2012; 42:1924–31. [PubMed: 22706795]
34. Saligan LN, Kim HS. A systematic review of the association between immunogenomic markers and cancer-related fatigue. Brain Behav Immun. 2012; 26:830–48. [PubMed: 22595751]
35. Whistler T, Taylor R, Craddock RC, Broderick G, Klimas N, Unger ER. Gene expression correlates of unexplained fatigue. Pharmacogenomics. 2006; 7:395–405. [PubMed: 16610950]
36. Gonzalez VJ, Tofthagen CS, Chen X, Pedro E, Saligan LN. Differences in Fatigue Severity in a Sample of Adult Cancer Patients. J Clin Nurs. 2017:05.

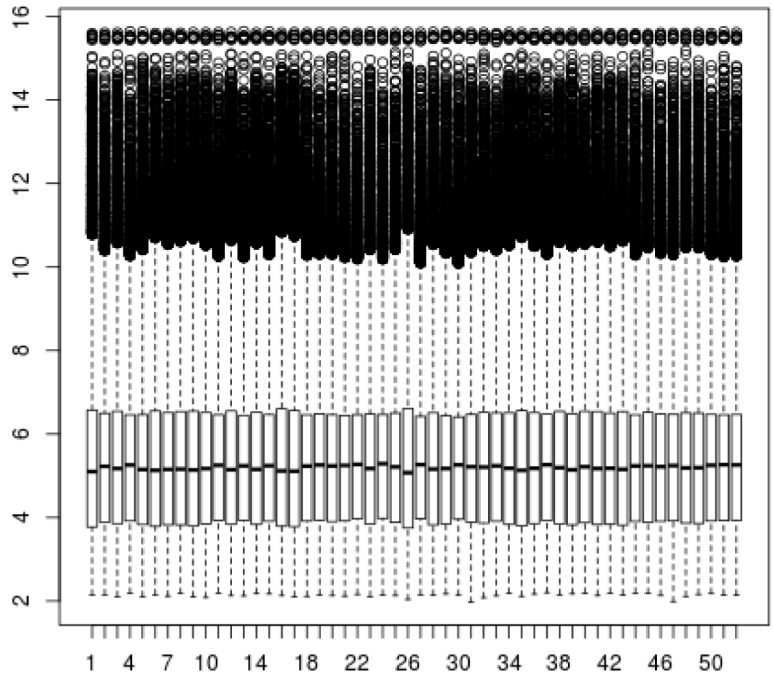

Figure 1. Boxplots of all Genes in each Array on the x-axis (n = 52 arrays), and the log base 2 of the Expression Values on the y axis

This figure assesses the quality of the 52 microarrays used in the study (along the x axis). Each array is represented as a box and whiskers plot summarizing the mean expression and quartiles for each array, in log base 2, along the y axis. The similar means (black bars) and first quartiles (white boxes) show that none of the arrays depart substantially from the mean expression, and all arrays may be retained with no further normalization required.

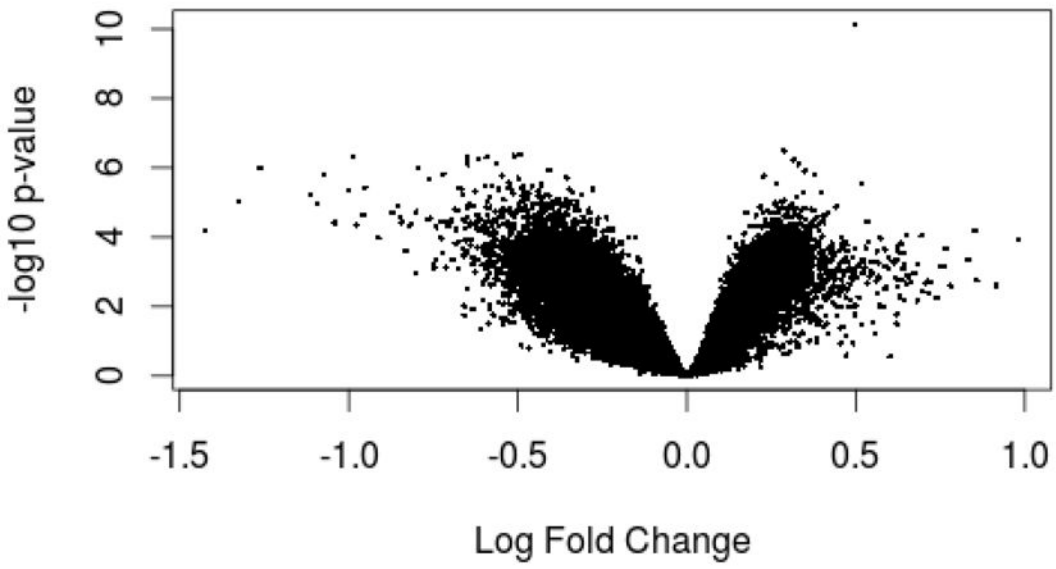

Figure 2. Volcano Plot Showing -log10 (p-value) versus fold changes in the y- and x-axis respectively of differentially expressed from baseline to midpoint of EBRT

A volcano plot summarizes the evidence of differential expression for each gene in the arrays. From a linear model fit of expression in midpoint versus baseline of EBRT we extract and plot the log base 2 ratios of gene expression (along the x axis) and the negative log base 10 of the p-value for each gene. Genes in the upper left quadrant of the volcano plot are downregulated at midpoint vs baseline (negative log ratios) and are differentially expressed (very small p-values); genes in the upper right quadrant are upregulated in the midpoint versus baseline of EBRT. Genes along the bottom of the plot have large p-values, with decreased evidence of differential expression.

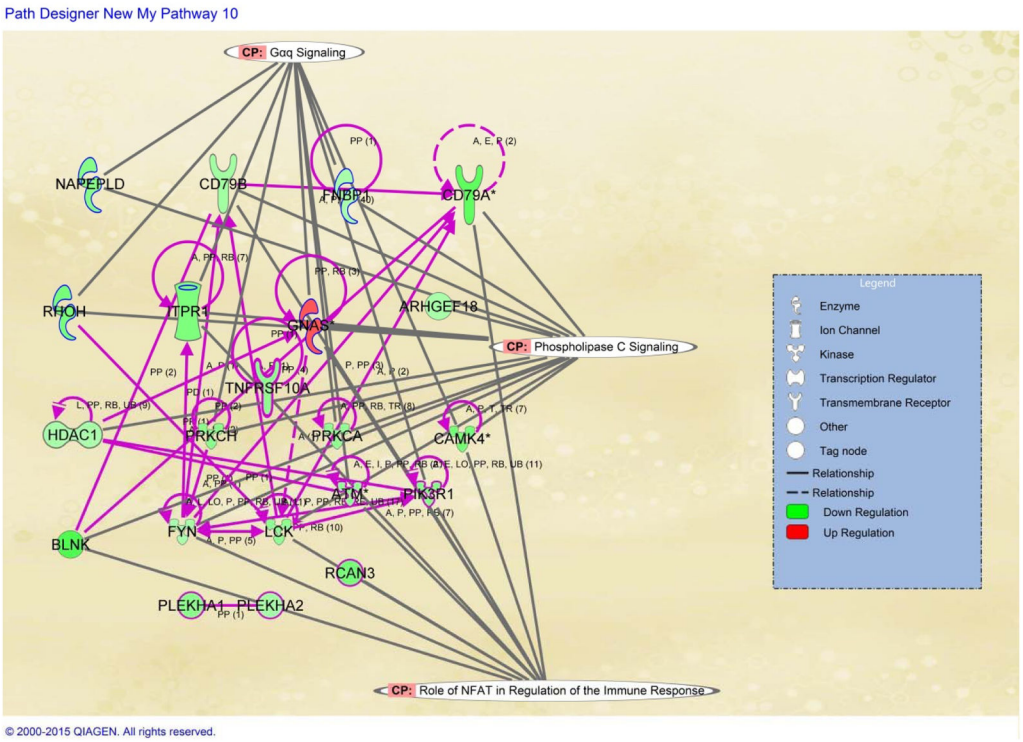

Figure 3. Top 3 Canonical Pathways of Radiotherapy-induced Gene Expression

A network of the top three canonical pathways (Phospholipase C Signaling, Role of NFAT in Regulation of the Immune Response, Gαq Signaling) and its associated differentially expressed genes was generated. Coloring is based on the expression values of the genes, down-regulation in green and, up-regulation in red. The direct and indirect relationships from the Ingenuity knowledge database are shown by solid and dashed lines, respectively. The arrow indicates specific directionality of interactions. *GNAS* was the only up-regulated gene common in the top three pathways. The canonical pathway is predominantly composed down-regulated genes; however, this is probably due to the fact that, although statistically significant, many of the up-regulated genes did not achieve the cutoff of 2-fold change.

Table 1

Demographic Characteristics of Study Participants

Characteristics	***N (%)***
Racial categories	
White	22 (85.0)
African American	4 (15.0)
Marital status	
Married	24 (92.3)
Single	1 (3.8)
Living together	1 (3.8)
Highest education completed	
Elementary/middle school	2 (7.7)
Some High School	2 (7.7)
High School Diploma	8 (30.8)
Some university no degree	1 (3.8)
Bachelor	7 (26.9)
Doctoral degree	3 (11.5)
Post-doctoral	3 (11.5)
Occupation	
Retired	18 (69.3)
Working	7 (26.9)
Handicap	1 (3.8)
Primary caregiver	
Wife	23 (88.5)
None	3 (11.5)

Table 2

Participants' Clinical Characteristics during Prostate Cancer Treatment

Variable	Mean	SD	Range	N	Reference Range
Gleason score (median)	7.00	.99	6–9	26	
PSA levels, ng/mL	5.42	4.34	.02–17.70	26	0–2.5 ng/mL
Hemoglobin	13.96	1.17	12.20–17.70	22	11–18g/dL
Albumin levels, g/dL	4.06	.28	3.4–4.5	16	3.4–5.0 g/dL
TSH, μIU/mL	2.68	1.33	1.18–5.27	7	0.465–4.68 μIU/mL
Number of RT Fractions (median)	42.00	2.10	38–43		
Number of RT Fields (median)	7.00	.39	6–8		

PSA= prostate specific antigen, TSH = thyroid stimulating hormone, RT = radiation therapy, ng= nanogram, mL= milliliter, dL = deciliter, μIU = micro international units

Table 3

Summary of Paired Samples t-test and Wilcoxon for the Functional Assessment of Cancer Therapy Fatigue subscale (FACT-F)

	Paired Samples T-test			Wilcoxon	
Pairs	***Mean (SD)***	***T***	***p-value***	***Z***	***p-value***
Baseline vs. Midpoint	42.38 (9.34) 42.11 (8.93)	.179	.859	−.66	.51
Baseline vs. End	42.38 (9.34) 43.03 (8.62)	−.389	.701	−.53	.60
Midpoint vs. End	42.11 (8.93) 43.03 (8.62)	−.791	.437	−1.14	.26

Table 4

Top 20 Up-regulated and Down-regulated Differentially Expressed Genes between Mid-point and Baseline based on Adjusted p value and Log Fold Change

Up-regulated Genes				Down-regulated Genes			
*S	Genes Symbol	Gene name	Expression value	*S	Genes Symbol	Gene name	Expression value
√	*XK*	X-linked Kx blood group (McLeod syndrome)	0.982 FC=1.97	√	*MS4A1*	membrane-spanning 4-do-mains, sub. fam A	−1.112 FC=0.46
	FGFR1OP2	FGFR1 oncogene partner 2	0.851 FC=1.80	√	*FCRLA*	Fc receptor-like A	−1.074 FC=0.74
	KLF1	Kruppel-like factor 1 (erythroid)	0.692 FC=1.61	√	*POU2AF1*	POU class 2 associating factor 1	−1.042 FC=0.48
	SESN3	sestrin 3	0.649 FC=1.56		*BANK1*	B-cell scaffold protein with ankyrin repeats 1	−1.039 FC=0.61
	ITLN1	intelectin 1 (galactofuranose binding)	0.533 FC=1.44	√	*IGHM*	immunoglobulin heavy constant mu	−0.999 FC=0.39
	FDXR	ferredoxin reductase	0.496 FC=1.41		*BACH2*	BTB and CNC homology 1, basic leucine zipper transcription factor 2	−0.989 FC=0.5
	DPM2	dolichyl-phosphate mannosyltransferase polypeptide 2, regulatory subunit	0.457 FC=1.37		*TCL1A*	T-cell leukemia/lymphoma 1A	−0.977 FC=0.53
	DPCD	deleted in primary ciliary dyskinesia homolog (mouse)	0.441 FC=1.35		*LINC00926*	long intergenic non-protein coding RNA 926	−0.957 FC=0.51
√	*RHD*	Rh blood group, D antigen	0.408 FC=1.32	√	*PAX5*	paired box 5	−0.952 FC=0.51
	ZER1	zyg-11 related, cell cycle regulator	0.406 FC=1.32	√	*CCR7*	chemokine (C-C motif) receptor 7	−0.856 FC=0.55

*S √ = is a gene found in a previous study/publication (Caucasian study, Saligan et al., 2013) N=20