Original Article

Proteomic Serum Profile of Fatigued Men Receiving Localized External Beam Radiation Therapy for Non-Metastatic Prostate Cancer

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Abstract

Context. Fatigue is the most distressing side effect of radiation therapy, and its progression etiology is unknown.

Objectives. This study describes proteome changes from sera of fatigued men with non-metastatic prostate cancer receiving external beam radiation therapy (EBRT).

Methods. Fatigue scores, measured by the Functional Assessment of Chronic Illness Therapy-Fatigue, and serum were collected from 12 subjects at baseline (before EBRT) and at midpoint (Day 21) of EBRT. Depleted sera from both time points were analyzed using two-dimensional difference gel electrophoresis, and up/down regulated proteins were identified using liquid chromatography-tandem mass spectrometry. Western blot analyses confirmed the protein changes observed.

Results. Results showed that apolipoprotein (Apo)A1, ApoE, and transthyretin (TTR) consistently changed from baseline (Day 0) to midpoint (Day 21). The mean ApoE level of subjects with high change in fatigue (HF: $n = 9$) increased significantly from baseline to midpoint and were higher than in subjects with no change in fatigue. The mean ApoA1 level was higher in HF subjects at baseline and at midpoint than in no fatigue subjects at both time points. The mean TTR level of no fatigue subjects was higher at baseline and midpoint than in HF subjects.

Conclusion. These ApoE, ApoA1, and TTR results may assist in understanding pathways that can explain fatigue progression etiology in this clinical population. J Pain Symptom Manage 2014;47:748–756. *Published by Elsevier Inc. on behalf of U.S. Cancer Pain Relief Committee.*

Key Words
External beam radiation therapy, fatigue, prostate cancer, quantitative proteomics, Western blot

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Introduction

Prostate cancer is the most common type of cancer and second leading cause of death among men in the U.S.[1] External beam radiation therapy (EBRT) is one of the treatment modalities preferred by patients with non-metastatic prostate cancer.[2] Up to 71% of prostate cancer patients complain of fatigue during EBRT, contributing to the decline of their health-related quality of life.[3,4] Fatigue related to cancer and its treatment, also known as cancer-related fatigue (CRF), is identified as the most distressing symptom reported by patients.[5] CRF is defined as a subjective feeling of overwhelming exhaustion that is not relieved by rest, interfering with the performance of a person's daily activities.[6] Evidence suggests that CRF can become a chronic condition post-cancer treatment, even impacting the quality of life of disease-free cancer survivors.[7] CRF remains poorly managed, and its etiology remains elusive.

Proteomic-based techniques have been used to identify several biomarkers to diagnose many types of cancer such as early-stage ovarian cancer,[8] prostate cancer,[9] gastric cancer,[10] and colorectal cancer.[11] These methods also help in identifying proteins that are involved in the underlying mechanisms of symptoms such as neuropathic pain,[12] depression,[13,14] and chronic fatigue.[15] Recently, one study used the surface-enhanced laser desorption/ionization (SELDI) technique, coupled with the use of one-dimensional gels and a trypsin digestion method using liquid chromatography, a proteomic methodology to identify possible biomarkers for CRF.[16] To our knowledge, no study has used the two-dimensional difference gel electrophoresis (2-D DIGE) technique to describe the changes in serum proteome that accompanies the clinically significant change in fatigue symptoms experienced by men with non-metastatic prostate cancer while receiving EBRT. This is an unbiased, hypothesis-generating approach, which can potentially identify proteomic markers that can explain the physiologic mechanisms behind the development or worsening of fatigue symptom in this clinical population.

Methods

Sample

Subjects with non-metastatic prostate cancer enrolled in an actively recruiting, National Institutes of Health (NIH), Institutional Review Board-approved protocol (NCT00852111) were included in this analysis. Subjects were 18 years or older, diagnosed with non-metastatic prostate cancer with or without a history of prostatectomy, and scheduled to receive EBRT with or without concurrent androgen deprivation therapy. Subjects with progressive disease causing significant fatigue; with psychiatric disease within five years; uncorrected hypothyroidism and anemia; taking sedatives, steroids, and nonsteroidal anti-inflammatory agents; and with second malignancies were excluded. The study was conducted at the Magnuson Clinical Research Center, NIH, Bethesda, MD, from May 2009 to September 2011. All participants signed written informed consent before participating in the study.

All study participants completed a fatigue questionnaire and had blood drawn at two time points: baseline (before EBRT) and at midpoint (Day 21 after EBRT initiation). Fatigue level was measured by the Functional Assessment of Chronic Illness Therapy-Fatigue (FACIT-F), a 13-item fatigue-specific subscale. The total score for the FACIT-F ranges from 0 to 52. A higher FACIT-F score indicates less fatigue. This is a validated fatigue measure, which showed good stability (test-retest $r = 0.87$) and good internal consistency reliability with a coefficient alpha in the mid-90s.[17] To optimize the phenotypic characterization of the study participants, subjects were grouped according to the change in fatigue scores during EBRT: the high fatigue (HF) group were subjects with increasing fatigue symptoms (declining FACIT-F scores) from baseline to midpoint of EBRT, and the no fatigue (NF) group were subjects with no change or with increasing FACIT-F scores between the two time points. Peripheral blood was collected using a serum separator tube (Becton, Dickinson and Company, Franklin Lakes, NJ) at both study time points. The sera were separated, aliquoted into separate Eppendorf tubes, and stored at $-80°C$ until ready for analysis.