

# Gene Expression Analysis of the mTOR Pathway: Association with Radiation Therapy–Related Fatigue

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**Objective:** To examine whether changes in the expression of 40 preselected mammalian target of rapamycin complex 1 (mTOR) pathway–related genes were associated with changes in fatigue scores during external beam radiation therapy (EBRT) to treat prostate cancer.

**Methods:** A total of 26 men completed the Functional Assessment of Cancer Therapy–Fatigue subscale and provided whole blood samples at baseline and at the midpoint of EBRT. Microarray gene expression data were analyzed using the limma package in R. Associations between changes in gene expression and changes in fatigue scores were computed using linear models and empirical Bayes moderation, with adjustment for radiation fraction.

**Results:** After adjusting for radiation fraction, changes in the expression of 24 of 40 preselected genes were significantly associated with changes in fatigue during EBRT (false discovery rate  $\leq .05$ ).

**Conclusion:** Findings suggest that mTOR-related genes may play a role in radiation therapy–related fatigue; validation of these implicated genes in an independent cohort is warranted.

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**Key words:** Cancer-related fatigue, mTOR signaling, Radiation therapy, Gene expression, Translational regulation

Radiation therapy (RT)–related fatigue is a significant clinical concern. It may affect up to 71% of prostate cancer (PC) patients being treated with external beam radiation therapy (EBRT), contributing to negative impacts on physical and emotional well-being and increasing the risk of mortality (1,2). This condition often leads to persistent, debilitating fatigue (~29% of PC patients) (3) and a potential economic burden related to loss of work, reduced productivity, and high healthcare costs for both patients and society. Therefore, improvements in the precision of symptom management and treatment are urgently needed and may be guided by the identification of novel underlying molecular mechanisms for cancer-related fatigue.

While current research on the biological underpinnings of fatigue during cancer treatment (e.g., immune dysregulation, hypothalamic–pituitary–adrenal axis dysregulation, mitochondrial dysfunction, gut–brain axis dysregulation) can guide precision oncology, this area of investigation remains a work in progress (4,5). Regarding peripheral mechanisms, we previously observed in whole blood samples from patients with PC that downregulation of the *SESN3* gene was associated with worsening fatigue during EBRT (6,7). The key role of *SESN3* in oxidative stress, inflammation, and tumorigenesis has been the subject of extensive reviews (8). Importantly, *SESN3* downregulation generally promotes mammalian target of rapamycin complex-1 (mTORC1 or mTOR) pathway activity by removing its inhibitory influence. This process disrupts energy balance and increases oxidative stress, which negatively affects the mitochondrial function (9). This mitochondrial impairment can lead to reduced adenosine

triphosphate production associated with fatigue development (10). A link between the mTOR pathway and exercise-induced fatigue has been proposed in an in vitro study (11). This proposed link highlights the importance of understanding the potential role of mTOR-related genes in RT-related fatigue, as they could serve as biomarkers and therapeutic targets. Therefore, this pilot study aims to build on our recent work by exploring whether, during EBRT for PC, changes in the expression of the preselected mTOR-related genes are associated with changes in Functional Assessment of Cancer Therapy–Fatigue (FACT-F) subscale scores. Our preliminary findings represent a first step toward identifying novel mTOR candidate genes that can be further validated in a new cohort.

## Methods

### Study population

This study was an exploratory, hypothesis-generating secondary analysis of previously collected gene expression microarray data

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(July–September 2014) from whole blood samples paired with phenotypic data from patients with PC (12,13). The dataset consists of 26 patients with a diagnosis of nonmetastatic PC who were scheduled to receive EBRT at an outpatient facility located in San Juan, Puerto Rico. Detailed inclusion and exclusion criteria have been published previously (7,12,13). Ethical approval for the study was obtained by a Midwest academic medical center (STUDY00001203) and by the University of Puerto Rico Medical Sciences Campus (protocol number: A6550113). Written informed consent was obtained from all participants, including consent for their data to be used in future research.

## Measures

Participants underwent 2 assessments—the first before EBRT initiation and the second at the midpoint of treatment (within 18 to 21 days after initiation)—in which they completed the FACT-F survey and provided blood samples. The midpoint was selected because prior studies in patients with PC identified this time point as the peak of fatigue (10). The Spanish version of the 13-item FACT-F questionnaire (scores range from 0 to 52, with higher scores representing less severe fatigue) has well-established validity and reliability in cancer patients (14).

For each of the 2 study time points, 2.5 mL of peripheral blood was collected into RNA PAXGene tubes (QIAGEN, Frederick, MD) and stored at the Alliance for Clinical and Translational Research laboratory in an ultra-low temperature (−80°C) freezer until shipment to the National Institute of Nursing Research (NINR) for RNA extraction. Briefly, at the NINR intramural laboratory of the National Institutes of Health, RNA extraction, purification, complementary DNA and complementary RNA synthesis, amplification, hybridization, scanning, and data analyses were performed following standard protocols (10,13). The detailed methodology of RNA extraction and microarray experiments was described previously (7,10,12,13). Fifty-two Affymetrix microarray chips (HG U133 Plus 2.0, Santa Clara, CA) were used to assess gene expression levels across the genome.

## Statistical analysis

Descriptive statistics were summarized for demographic characteristics, clinical variables, and fatigue scores. Gene expression analyses were based on previously generated Affymetrix microarray data (12,13). In the original genome-wide analysis, gene expression intensity data were normalized using the limma package in R, and associations between changes in gene expression and fatigue scores were evaluated using linear models with empirical Bayes moderation, adjusting for either radiation fraction or the total number of days of prescribed radiation treatment (7,12,13). Specifically, changes in gene expression from baseline to the midpoint of EBRT were evaluated in relation to changes in FACT-F scores over the same period, while accounting for radiation fraction. The Benjamini–Hochberg method was applied to account for multiple testing across thousands of genes by estimating the false discovery rate (FDR), as described in prior publications (7,10,12,13).

For the present secondary exploratory analysis, we focused on a biologically prespecified set of 40 mTOR-related genes

identified from existing literature (15–17). This included the mTOR signaling pathway gene set reported by Passtoors et al. (15), which demonstrated differential expression of mTOR pathway genes associated with human longevity. Additional studies have implicated mTOR pathway dysregulation in cancerous and noncancerous conditions (16), providing a biological rationale for examining changes in pathway expression in relation to fatigue during EBRT for PC. The 40 preselected mTOR-related genes were manually extracted from the original genome-wide differential expression results. Genes with an FDR  $\leq 0.05$  were considered statistically significant.

## Results

Participant demographic and clinical characteristics are presented in Table 1. Overall, study participants ( $n = 26$ ) had a mean age of 67.01 years (SD = 7.56); 96% were partnered, and 84%

**Table 1.** Study participant demographic and clinical characteristics

| Characteristics                    | N (%)        |    |
|------------------------------------|--------------|----|
| <i>Marital status</i>              |              |    |
| Married or partnered               | 25 (96)      |    |
| Single                             | 1 (4)        |    |
| <i>Education</i>                   |              |    |
| Elementary/middle school           | 2 (8)        |    |
| Some high school                   | 2 (8)        |    |
| High school diploma                | 8 (30)       |    |
| Some university, no degree         | 1 (4)        |    |
| Bachelor's degree or higher        | 13 (50)      |    |
| <i>Occupation</i>                  |              |    |
| Retired                            | 18 (70)      |    |
| Working                            | 7 (26)       |    |
| Handicapped                        | 1 (4)        |    |
| Clinical characteristics           | N (%)        |    |
| <i>Tumor stage</i>                 |              |    |
| T1c                                | 9 (35)       |    |
| T2b                                | 12 (46)      |    |
| T3                                 | 4 (15)       |    |
| T4                                 | 1 (4)        |    |
| <i>EBRT prescription</i>           |              |    |
| 43 fractions with 77.4 Gy          | 14 (54%)     |    |
| 42 fractions with 75.6 Gy          | 6 (23%)      |    |
| 38 fractions with 68.4 Gy          | 5 (19%)      |    |
| 38 fractions with 61.2 Gy          | 1 (4)        |    |
|                                    | Mean (SD)    | N  |
| Gleason score (median)             | 7.00         | 26 |
| Body mass index, kg/m <sup>2</sup> | 29.39 (4.16) | 26 |
| PSA at baseline, ng/mL             | 5.42 (4.34)  | 26 |
| Hemoglobin, g/dL                   | 13.96 (1.17) | 22 |
| TSH, μIU/mL                        | 2.68 (1.33)  | 7  |

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

had a high school or higher education. All participants received EBRT via the intensity-modulated RT technique; of these, the largest percentage (54%) received a total of 43 fractions with 77.4 Gy, 42% had received concurrent neoadjuvant hormonal therapy (androgen deprivation therapy [ADT]) at the midpoint of EBRT, and 23% had undergone radical prostatectomy more than a year before receiving EBRT. The mean fatigue score before treatment was 42.38 (SD = 9.34), and the mean score at the midpoint was 42.11 (SD = 8.93). There were no significant differences in fatigue across time between those on ADT and those not on ADT ( $P > .05$ ). More specifically, we compared the results of the changes in fatigue (baseline minus midpoint) among those who received concomitant ADT ( $n = 11$ ) with those who did not ( $n = 15$ ). The t-test showed that there were no significant differences in fatigue changes between those on ADT and those not on ADT ( $t(24) = -.45$ ,  $P = .66$ ). Additionally, there was no significant correlation between changes in fatigue (baseline minus midpoint) and participant age (Spearman  $\rho = .23$ ,  $P = .25$ ). After FDR correction ( $FDR \leq .05$ ), approximately 60% (24 of 40) of the preselected genes showed differential expression of at least 1 probe, which was significantly associated with changes in fatigue ( $\Delta\text{FACT-F}$  scores) across all study participants after adjusting for radiation fraction (Table 2). Figure 1 summarizes the genes that were significantly associated with changes in fatigue during EBRT after adjusting for radiation fraction ( $FDR \leq .05$ ). More specifically, Figure 1 illustrates the significant associations identified among probes of the 24 genes. These genes represent multiple functional branches of the mTOR pathway, including an essential core component of the mTORC1 complex, upstream regulation of mTOR signaling, mTOR activation, negative regulation of mTORC1 signaling, translational control, stress-response pathways, and mitochondrial function.

## Discussion

The relationship of changes in the mTOR signaling pathway at the level of gene expression can provide insight into potential mechanisms behind the development of RT-related fatigue and may provide molecular targets for individualized interventions, leading to more effective management of fatigue in this population. Differential expression of all the probes for 10 mTOR-related genes was significantly upregulated and associated with changes in fatigue ( $FDR \leq .05$ ). For example, our results identified genes that are downstream effectors and targets of the mTOR pathway (e.g., *EIF4B*, *RPS6KB1*) and genes involved in various cellular processes, including mitochondrial biogenesis (*YY1*), stress resistance (*FOXO1*), and PI3K/AKT/mTOR pathway signaling (*PIK3R1*). In fact, both *EIF4B* and *RPS6KB1* (*S6K1*) are key downstream targets of the mTOR pathway. These targets are crucial for regulating protein synthesis, cell growth, and proliferation, with mTOR directly phosphorylating and activating these downstream proteins to promote translation initiation (17). Notably, most of these differentially expressed genes are not well described in the cancer-related fatigue literature. In support of our findings, an animal study reported that central fatigue is directly related to an increase in *p70S6K* (18). Additionally, *PIK3R1* upregulation was identified in the blood of patients with immune-

related fatigue conditions—such as chronic fatigue syndrome (19) and Sjögren syndrome (20)—a finding that may relate to our results. The identification of transcription factors associated with mitochondrial biogenesis and stress adaptation (*YY1*, *FOXO1*, and *FOXO3*) may highlight the multifaceted role of mTOR in mitochondrial function and cellular stress responses—both of which are also implicated in fatigue development (4,10,21).

Eleven of the downregulated genes associated with changes in fatigue are involved in key regulatory zones of the mTOR pathway. These genes include an essential core component of the mTORC1 complex (*RPTOR*); an mTOR upstream regulator (*MAPK1*) or activator (*RHEB*); negative regulators (*AKT1S1*, *FKBP8*, and *FKBP1B*); translational or downstream effectors (*EIF4E*, *POLDIP3*, and *RPS6KL1*); and genes driving various cellular processes, including the cell cycle (*CDKN2C*) and stress responses (*FOXO3*). In contrast to our findings, upregulation (rather than downregulation) of *RHEB* has been associated with volitional fatigue (21). Additionally, while *VEPH1* has been identified as being downregulated in men with PC ( $n = 43$ ) who experienced fatigue 1 year after EBRT (22), *VEPH1* expression did not reach the threshold for biological significance in our cohort ( $FDR < .05$ ). Interestingly, we found that *FOXO*, *4EBP*, *AKT1*, and *MAPK1* are known targets of herbal medicines being evaluated for their ability to ameliorate symptoms such as fatigue (23,24), suggesting opportunities for future interventional research. While the relationships between most of these genes and fatigue have yet to be validated, higher mTOR expression is observed in PC tumors and has been associated with disease progression (16).

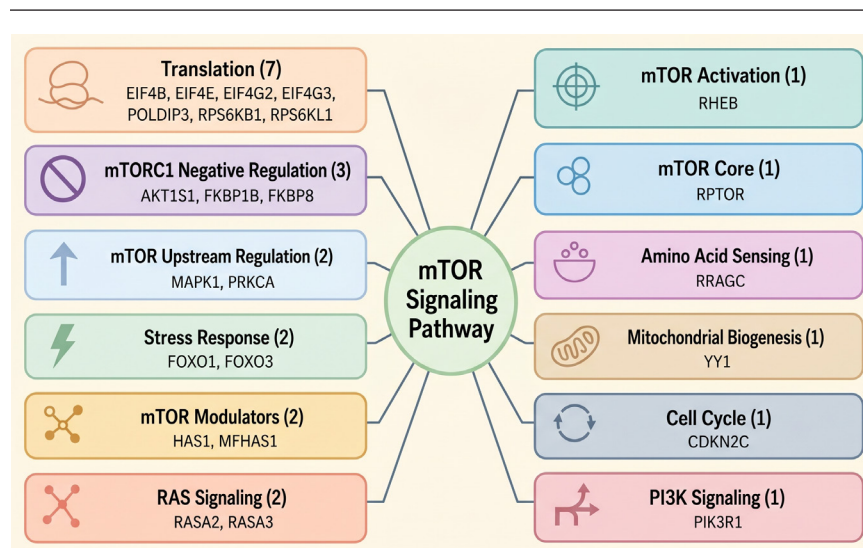
The results should be interpreted with caution in light of the previously mentioned study limitations (7,12,13), the most prominent being the sample size ( $n = 26$ ), which restricted our ability to adjust for additional clinical treatment variables, such as ADT and prior prostatectomy. Although no significant differences in these characteristics were observed between fatigue groups, larger studies are needed to determine potential confounding effects of these clinical variables, as well as the influence of other, unmeasured factors (e.g., anxiety, stress). Although rigorous quality control, normalization, and multiple-testing correction procedures were applied, this exploratory secondary analysis was conducted in a small cohort without technical replication or external validation. The goal of this analysis was to identify candidate mTOR-related genes associated with fatigue rather than establish definitive biomarkers; therefore, these findings require confirmation in larger independent studies using complementary transcriptomic approaches, as the current study is unable to draw causal conclusions despite identifying significant differentially expressed genes associated with fatigue. We did not determine gene expression associations at the end of or beyond EBRT; thus, our findings provide a snapshot at the midpoint of EBRT. Although microarray analysis was an appropriate genome-wide gene expression approach at the time of data collection (2014), our findings should be replicated/validated, perhaps using more sensitive technologies capable of detecting low-abundance transcripts (e.g., RNA sequencing), in a large sample that would include different treatment time points to observe whether these differentially expressed genes, along with other phenotypic characteristics, predict persistent fatigue.

**Table 2.** Associations between changes in 40 pre-selected mTOR-related gene expression levels and changes in fatigue from baseline to the midpoint of external beam radiation therapy (N = 26)

| Gene symbol | Functional category        | Probe ID     | RT_Fraction <sup>a</sup> | $\beta$ ( $\Delta$ Fatigue) <sup>b</sup> | Average expression <sup>c</sup> | F value | P value | FDR   |
|-------------|----------------------------|--------------|--------------------------|------------------------------------------|---------------------------------|---------|---------|-------|
| MTOR        | mTOR core                  | 215381_at    | -0.006                   | -0.007                                   | -0.225                          | 4.524   | .019    | .075  |
| MTOR        |                            | 202288_at    | -0.002                   | 0.001                                    | -0.084                          | 1.894   | .168    | .293  |
| RPTOR       |                            | 225715_at    | -0.016                   | -0.004                                   | -0.650                          | 8.601   | .001    | .014* |
| MAPKAP1     | mTOR upstream regulation   | 229846_s_at  | 0.005                    | 0.011                                    | 0.214                           | 5.318   | .011    | .052  |
| MAPKAP1     |                            | 222426_at    | 0.004                    | 0.018                                    | 0.153                           | 5.243   | .011    | .054  |
| MAPK1       |                            | 224620_at    | -0.002                   | -0.016                                   | -0.085                          | 6.028   | .006    | .039* |
| MAPK1       |                            | 229847_at    | -0.002                   | 0.011                                    | -0.082                          | 5.543   | .009    | .047* |
| PRKCA       |                            | 213093_at    | 0.012                    | 0.017                                    | 0.512                           | 15.651  | .001    | .002* |
| PRKCA       |                            | 206923_at    | -0.006                   | -0.005                                   | -0.264                          | 9.026   | .001    | .012* |
| PRKCA       |                            | 1560074_at   | -0.003                   | -0.010                                   | -0.104                          | 3.748   | .035    | .108  |
| RHEB        | mTOR activation            | 201451_x_at  | -0.003                   | -0.010                                   | -0.138                          | 3.846   | .033    | .103  |
| RHEB        |                            | 241795_at    | -0.006                   | 0.005                                    | -0.237                          | 6.108   | .006    | .037* |
| TIRAP       |                            | 1552360_a_at | 0.005                    | 0.006                                    | 0.201                           | 5.073   | .013    | .058  |
| AKT1        |                            | 207163_s_at  | 0.002                    | -0.006                                   | 0.089                           | 1.541   | .231    | .362  |
| RRAGC       | Amino acid sensing         | 242531_at    | -0.005                   | 0.003                                    | -0.200                          | 7.634   | .002    | .020* |
| RRAGC       |                            | 218088_s_at  | 0.005                    | -0.003                                   | 0.190                           | 6.628   | .004    | .030* |
| RRAGA       |                            | 201628_s_at  | 0.003                    | 0.007                                    | 0.122                           | 1.895   | .168    | .293  |
| RRAGB       |                            | 205540_s_at  | <.001                    | 0.003                                    | 0.001                           | 0.097   | .908    | .932  |
| RRAGD       |                            | 221523_s_at  | 0.001                    | 0.011                                    | 0.012                           | 0.215   | .808    | .857  |
| RRAGD       |                            | 221524_s_at  | <.001                    | 0.003                                    | 0.003                           | 0.075   | .928    | .947  |
| AKT1S1      | mTORC1 negative regulation | 224982_at    | -0.007                   | 0.002                                    | -0.278                          | 5.720   | .008    | .044* |
| AKT1S1      |                            | 1555821_a_at | -0.003                   | 0.005                                    | -0.156                          | 4.283   | .023    | .084  |
| FKBP8       |                            | 222007_s_at  | -0.005                   | -0.021                                   | -0.187                          | 6.862   | .004    | .027* |
| FKBP1B      |                            | 206857_s_at  | -0.015                   | 0.021                                    | -0.613                          | 14.777  | .001    | .002* |
| TSC1        |                            | 209390_at    | 0.004                    | 0.006                                    | 0.137                           | 5.267   | .011    | .053  |
| TSC2        |                            | 215624_at    | -0.001                   | -0.015                                   | -0.073                          | 3.026   | .063    | .150  |
| TSC2        |                            | 215735_s_at  | 0.001                    | -0.007                                   | 0.046                           | 0.889   | .421    | .540  |
| DEPTOR      |                            | 234071_at    | -0.003                   | -0.011                                   | -0.118                          | 3.162   | .057    | .146  |
| EIF4B       | Translation                | 211938_at    | 0.010                    | 0.016                                    | 0.386                           | 9.965   | .001    | .010* |
| EIF4B       |                            | 211937_at    | 0.007                    | 0.006                                    | 0.305                           | 8.154   | .002    | .017* |
| EIF4B       |                            | 219599_at    | 0.006                    | 0.017                                    | 0.253                           | 7.407   | .002    | .022* |
| EIF4E       |                            | 237718_at    | -0.005                   | -0.004                                   | -0.198                          | 6.674   | .004    | .030* |
| EIF4E       |                            | 201437_s_at  | 0.008                    | 0.030                                    | 0.311                           | 5.061   | .013    | .058  |
| EIF4E       |                            | 201435_s_at  | 0.005                    | 0.025                                    | 0.178                           | 2.290   | .119    | .233  |
| EIF4EBP1    |                            | 221539_at    | -0.002                   | 0.007                                    | -0.104                          | 4.516   | .019    | .075  |
| EIF4G3      |                            | 201935_s_at  | 0.008                    | -0.018                                   | 0.321                           | 7.167   | .003    | .024* |
| EIF4G2      |                            | 200004_at    | 0.010                    | 0.013                                    | 0.413                           | 6.706   | .004    | .029* |
| POLDIP3     |                            | 210583_at    | -0.007                   | -0.005                                   | -0.270                          | 9.418   | .001    | .011* |
| POLDIP3     |                            | 243411_at    | -0.004                   | -0.016                                   | -0.158                          | 7.063   | .003    | .025* |
| POLDIP3     |                            | 212100_s_at  | 0.003                    | -0.002                                   | 0.114                           | 3.054   | .062    | .154  |
| RPS6        |                            | 211690_at    | -0.002                   | -0.005                                   | -0.078                          | 1.508   | .238    | .369  |
| RPS6KL1     |                            | 223534_s_at  | -0.006                   | -0.007                                   | -0.242                          | 8.385   | .001    | .016* |
| RPS6KB1     |                            | 204171_at    | 0.005                    | 0.023                                    | 0.212                           | 6.733   | .004    | .029* |
| RPS6KB1     |                            | 226660_at    | 0.007                    | 0.055                                    | 0.261                           | 5.641   | .008    | .045* |
| YY1         | Mitochondrial biogenesis   | 224711_at    | 0.009                    | 0.012                                    | 0.380                           | 14.172  | <.001   | .003* |
| YY1         |                            | 224718_at    | 0.008                    | 0.002                                    | 0.313                           | 11.780  | .001    | .005* |
| YY1         |                            | 201901_s_at  | 0.007                    | -0.002                                   | 0.277                           | 9.355   | .001    | .011* |
| YY1         |                            | 201902_s_at  | 0.001                    | 0.002                                    | 0.016                           | 0.044   | .957    | .968  |
| CDKN2C      | Cell cycle                 | 204159_at    | -0.001                   | -0.001                                   | -0.049                          | 0.150   | .861    | .898  |
| CDKN2C      |                            | 211792_s_at  | -0.006                   | 0.006                                    | -0.253                          | 6.298   | .005    | .034* |
| FOXO1       | Stress response            | 228484_s_at  | -0.002                   | -0.011                                   | -0.010                          | 1.749   | .191    | .320  |
| FOXO1       |                            | 202723_s_at  | 0.009                    | -0.003                                   | 0.366                           | 15.074  | <.001   | .002* |
| FOXO1       |                            | 202724_s_at  | 0.012                    | 0.023                                    | 0.473                           | 14.650  | <.001   | .002* |
| FOXO3       |                            | 224889_at    | -0.002                   | -0.012                                   | -0.060                          | 1.348   | .275    | .407  |
| FOXO3       | mTOR modulators            | 231548_at    | -0.005                   | -0.007                                   | -0.207                          | 5.544   | .009    | .047* |
| MFHAS1      |                            | 225478_at    | 0.007                    | 0.009                                    | 0.276                           | 7.380   | .003    | .022* |
| HAS1        | PI3K signaling             | 207316_at    | -0.006                   | -0.002                                   | -0.244                          | 6.167   | .006    | .036* |
| VEPH1       |                            | 229759_s_at  | -0.004                   | -0.008                                   | -0.177                          | 5.167   | .012    | .056  |
| VEPH1       |                            | 232122_s_at  | -0.003                   | -0.007                                   | -0.138                          | 3.982   | .029    | .097  |
| SMAD1       |                            | 227798_at    | -0.001                   | 0.029                                    | -0.013                          | 4.809   | .015    | .065  |
| PIK3R1      | PI3K signaling             | 212239_at    | 0.013                    | -0.001                                   | 0.567                           | 15.295  | <.001   | .002* |
| PIK3R1      |                            | 212240_s_at  | 0.011                    | 0.006                                    | 0.454                           | 13.305  | <.001   | .003* |
| PIK3R1      |                            | 212249_at    | 0.011                    | 0.010                                    | 0.458                           | 10.536  | .001    | .008* |
| RASA3       | RAS signaling              | 225562_at    | 0.007                    | 0.009                                    | 0.307                           | 9.329   | .001    | .011* |
| RASA2       |                            | 206636_at    | 0.006                    | 0.009                                    | 0.269                           | 5.724   | .008    | .044* |
| RASA2       |                            | 230669_at    | 0.008                    | -0.001                                   | 0.335                           | 14.370  | <.001   | .003* |
| ULK1        | Autophagia                 | 209333_at    | -0.001                   | -0.010                                   | -0.013                          | 0.841   | .441    | .562  |
| HIF1A       | Angiogenesis               | 200989_at    | 0.005                    | 0.021                                    | 0.193                           | 1.516   | .236    | .368  |
| SREBF1      | Lipid synthesis            | 202308_at    | 0.001                    | -0.012                                   | 0.040                           | 3.518   | .042    | .121  |
| SREBF1      |                            | 1558875_at   | -0.001                   | -0.009                                   | -0.043                          | 0.950   | .398    | .524  |

Abbreviations: FDR, false discovery rate; RT, radiation therapy. <sup>a</sup>Coefficient estimate for the variable RT fraction based on the fitted model. <sup>b</sup>Coefficient estimate for the delta fatigue (based on the fitted model). <sup>c</sup>Average log2 expression for the probe over all arrays. \* Significant association between change in gene expression and change in fatigue at FDR  $\leq$  .05.

**Figure 1.** A summary of the genes that were significantly associated with the changes in fatigue scores during external beam radiation therapy after adjusting for radiation fraction (FDR  $\leq .05$ ), and their mTOR-related functions



Note. Only genes remaining significant after Benjamini-Hochberg false discovery rate correction (FDR  $\leq .05$ ) are named. The diagram provides a conceptual summary of the functional organization of genes significantly associated with fatigue during external beam radiation therapy and is intended to facilitate interpretation of the principal findings rather than depict a complete mechanistic signaling pathway.

Abbreviations: EBRT, external beam radiation therapy; FDR, false discovery rate; mTOR, mechanistic target of rapamycin; mTORC1, mechanistic target of rapamycin complex 1; PI3K, phosphatidylinositol 3-kinase; RAS, RAS protein family.

## Conclusion

We identified a novel set of mTOR-related candidate genes associated with RT-related fatigue, suggesting that the mTOR pathway may play a role in such fatigue. We are currently conducting the next phase of this research (grant R21CA288925), initially validating the expression of specific mTOR-implicated genes using RNA sequencing in an independent sample. Our long-term goal is to identify molecular biomarkers and modifiable social determinants of health that can guide diagnostic precision and refine therapeutic strategies for cancer-related fatigue.

## Resumen

**Objetivo:** Examinar si los cambios en la expresión de 40 genes preseleccionados relacionados con la vía del complejo 1 de la diana de la rapamicina en mamíferos (mTOR) estaban asociados con cambios en las puntuaciones de fatiga durante la radioterapia para tratar el cáncer de próstata. **Métodos:** Veintiséis hombres completaron el cuestionario de la subescala de fatiga de la Evaluación Funcional de la Terapia del Cáncer y proporcionaron muestras de sangre al inicio y a la mitad de la radioterapia. Los datos de expresión génica se analizaron utilizando el paquete limma en R. Las asociaciones se calcularon mediante modelos lineales y moderación empírica bayesiana, ajustados por la fracción de radiación. **Resultados:**

Tras ajustar por la fracción de radiación, los cambios en la expresión de 24 de los 40 genes preseleccionados se asociaron significativamente con los cambios en la fatiga (tasa de falso descubrimiento  $< .05$ ). Conclusión: Los hallazgos sugieren que los genes relacionados con mTOR podrían desempeñar un rol en la fatiga asociada a la radioterapia; se justifica la validación de estos hallazgos en una cohorte independiente.

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