Overexpression of TIGAR and HO-1 in peripheral blood mononuclear cells (PBMCs) of breast cancer patients treated with radiotherapy

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Abstract

Introduction: Radiation therapy (RT) is one of the primary treatment choices for breast cancer. In reaction to RT, many metabolic processes in the body are triggered, some of which have a role in counteracting free radicals in cancer cells. As a result, it is important to comprehend the effects of RT on multiple genes, biomarkers and enzymes in the body.

Methods and materials: Peripheral blood mononuclear cells (PBMCs) were obtained from 83 breast cancer patients in pre-and post-RT (50 Gray (Gy) in 25 fractions). The TIGAR and HO-1 gene expressions were investigated by quantitative real-time PCR (qRT-PCR). Serum bilirubin, total antioxidant capacity (TAC), total protein (TP), alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were assayed in serum patients before and after RT.

Results: We found that bilirubin (p = .001), ALT (p = .04), and AST (p = .03) were significantly increased, while TAC (p < .001) and TP (p = .001) were decreased after RT. However, albumin and ALP did not change after RT (p > .05 for both). Interestingly, RT led to overexpression of TIGAR (p = .004) and HO-1 (p = .003) genes in breast cancer patients.

Conclusions: The findings of this study showed that RT could overexpress TIGAR and HO-1 in PBMCs of breast cancer patients. More research is required to figure out the mechanisms behind the impacts of RT on increased catabolism and production of bilirubin or increased activity of TIGAR-related pathways and overexpression of TIGAR and HO-1.

Abbreviations: RT: Radiotherapy; ROS: Reactive Oxygen Species; HO-1: Heme Oxygenase-1; TIGAR: Tp53-Induced Glycolysis; PBMC: Peripheral Blood Mononuclear Cells; TAC: Total Antioxidant Capacity; TP: Total Protein; AST: Aspartate Transaminase; ALT: Alanine Transaminase; ALP: Alkaline Phosphatase; ER: Estrogen Receptor; PR: Progesterone Receptor; HER2: Human Epidermal Growth Factor Receptor 2.

Introduction

With an estimated 2.3 million new cases, breast cancer is now the leading cause of global cancer incidence in 2020 (DeSantis et al. 2019; Sung et al. 2021). In recent years, significant progress has been made in understanding potential cancer therapeutic hallmarks. Generally, chemotherapy, hormone therapy, and radiotherapy (RT) are commonly used to treat cancer patients (Acevedo et al. 2014; Klein et al. 2019; Plym et al. 2020). Among these treatments, RT is an important part of cancer treatment, with nearly half of all cancer patients receiving it at some period during their disease, and it accounts for 40% of cancer curative treatment. The primary objective of RT is to deprive cancer cells of their ability to multiply (cell division) (Baskar et al. 2012; Haussmann et al. 2020). However, it is suggested that generating cytotoxic reactive oxygen species (ROS) RT affects both tumor cells and normal cells due to producing the ROS that is able to damage virtual cellular macromolecules such as DNA (deoxyribonucleic acid), RNA (ribonucleic acid), microRNAs, proteins and cell membrane (Marin et al. 2015). Moreover, RT has the potential to alter the status of the antioxidant/oxidant system (Arjmandi et al. 2016; Sisakht et al. 2020). For example, heme oxygenase (HO) by heme degradation, leads to the formation of bilirubin, carbon monoxide (CO), and free iron, which plays crucial roles in defense against oxidative stress and cellular stress (Maines 1988; Shi and Fang 2008). Moreover, the over-expression of HO-1 results in an increase in the free ions that can induce cell damage and also an increase in serum bilirubin that should exert from urine (Duvigneau et al. 2008). The turn-over of heme oxygenase protein is mediated by ubiquitin proteasome system (UPS) (Lin et al. 2008) and the components of this system are also involve in tumorigenesis of...
breast cancer (Seghatoleslam et al. 2012). TP53-induced glycolysis and apoptosis regulator (TIGAR) is a p53 target protein that plays a significant role in glycolysis and redox system. TIGAR is highly expressed in cancer cells. It can consequently induce redirection of glycolysis to the pentose phosphate pathway (PPP) (Geng et al. 2018). This can promote tumor cell growth by providing metabolic intermediates and reductive substrates derived from PPP. The expression of TIGAR in cancer cells is positively interlinked with chemotherapy resistance, suggesting that TIGAR could be a novel therapeutic target in cancer treatment (Geng et al. 2018).

Breast cancer patients treated with RT can be exposed to a variety of cellular and molecular damage via the production of ROS. The impact of RT on gene expression, however, among breast cancer patients has not been fully examined and limited evidence is existing on this topic. This study investigated the differential expression of TIGAR and HO genes before and after RT in PBMCs of patients with breast cancer. Furthermore, some biochemical factors such as serum bilirubin, total antioxidant capacity (TAC), total protein (TP) alanine aminotransferase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) were assessed in patients’ serum, before and after RT.

**Material and methods**

**Participants**

The Shiraz University of Medical Sciences Ethics Committee has approved the study, and informed consent was obtained from all participants (approval code: 21117-01-01-98). From April 2019 to January 2020, patients that were referred to the radio-oncology department in Nemazee hospital for 9 months were examined to be included in the study. Patients were included if they were newly pathologically diagnosed with breast cancer (not be a relapse or recurrence case), and also if prior to RT, patients did not receive chemotherapy. Patients who suffered from any other cancer or liver diseases were excluded from the study.

**Samples**

Then blood samples were collected from patients before and 3 weeks after the RT. The whole-blood samples were collected with and without an anticoagulant agent (EDTA) separately. The fresh whole blood samples were used for RNA extraction from whole white blood cells. Serum samples were separated from whole blood (2000 rpm) and kept refrigerated (4°C) for the measurement of bilirubin, TP, albumin, ALT, AST, ALP, and TAC. The study follows the diagram presented in Figure 1.

**Peripheral blood mononuclear cells (PBMC) extraction**

The fresh blood samples were diluted with phosphate-buffered saline (PBS; 1:1 ratio) and added to 3 mL of Ficoll solution (Sigma-Aldrich). Then, they were mixed gently and were centrifuged for 20 minutes at 2500 rpm. The PBMC layer was separated from the red blood cells, granulocytes, and plasma layers. They were then rinsed and centrifuged
three times with PBS Ribonucleic Acid Extraction (each time for 10 minutes at 200 g) (Ulmer et al. 1984).

Total ribonucleic acid (RNA) was prepared from the whole white blood cells (WBC) samples (RNA extraction kit (RNX-Plus, Sinaclon)) according to the manufacturer’s instruction. The RNA concentration was calculated by nanodrop. The OD260/OD280 ratio and gel agarose electrophoresis (2%) were used to determine RNA quantity and quality.

**Complementary deoxyribonucleic acid synthesis**

According to the manufacturer’s instructions, complementary deoxyribonucleic acid (cDNA) was synthesized using a cDNA Synthesis kit (Prime Script II strand cDNA Synthesis Kit, euroX. Poland).

**Quantitative real-time polymerase chain reaction**

The TIGAR and HO gene expression levels were measured by SYBR Green quantitative (q) - Real-Time PCR technique (RG-6000 Rotor-Gene, Corbett Research, Sydney, Australia) and were normalized with the Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH)) gene. The amplification reaction was carried out in a volume of 10 μL utilizing forward and reverse primers (0.5 μm) and a cDNA sample (1 μL).

The q-RT-PCR program consisted of an initial denaturation stage of 95°C at 3 minutes. Then, a three-step program was developed for 40 cycles including denaturation at 95°C for 10 seconds, annealing at 61°C, 60°C, 60°C for GAPDH, TIGAR, and HO, (all for the 20 s) respectively, and 72°C for 20 seconds, followed by a final extension step at 72°C for 5 min. The 2-ΔΔCt method was used to determine the relative quantity of the target genes. To ensure the purity of each reaction’s amplification product, a melting curve analysis was generated for each product. (Zhang et al. 2017).

The primers (Table 1) were created using Oligo 6.0 (Molecular Biology Insights, Cascade, CO, USA) and verified using the primer blast (NCBI).

**Biochemical factors**

Biochemical serum factors such as bilirubin, ALT, AST, TP, and albumin of the patients before and after the RT were measured using standard kits (Pars Azmoun Co., Tehran, Iran) and an Auto Analyzer (BT3000, Italy) (Rahimi-Madiseh et al. 2017).

**Total antioxidant capacity (TAC) measurement**

The ferric reducing ability of plasma (FRAP) test was used to analyze TAC. In this test, the conversion of ferric to ferrous ions in the presence of antioxidants at a low pH results in the formation of a colorful complex called Fe (II) -TPTZ (Ferrous-Tri-pyridyl-Striazine), with a maximum absorbance of 593 nm (Benzie and Strain 1996). The variations in absorbance in serum samples and standards were measured and represented in mol/L.

**Statistical analysis**

The demographic and pathologic data of patients were collected using hospital records and a valid questionnaire. Statistical analyzes were performed using a statistical software package (SPSS, version 22.0, SPSS Inc., Chicago, IL). Normality of data was checked by normality plot with test and the parametric data of at least three independent experiments were reported as mean ± standard deviation (SD) and were evaluated with paired sample t-test and analysis of variance (ANOVA) test. Moreover, correlations were checked by using Pearson correlation (r) with 2-tails of significance. p Value less than .05 was considered significant.

**Results**

**Participant characteristics**

As shown in Figure 1, a total of 210 breast cancer patients were examined to find out if they met the study inclusion criteria. Accordingly, 35 patients were excluded (for the following reasons: 15 with relapse, 11 suffer from other cancers, 9 with liver disease) and then 9 patients were dropped as a result of losses to follow up. Then, we collected 158 intact blood samples before and 158 intact blood samples after RT, of the 83 intact RNA and gene expression (83 intact RNA and gene expression before and 83 intact RNA and gene expression after RT) were examined.

Table 2 shows the basic characteristics and pathologic information of 83 breast cancer participants. Among 83 women with breast cancer who participated in this study, 29 (35%) were younger than 50 years of age. The patients were on average 55.86 (SD = 9.38) years old. The average patients’ body mass index (BMI) was 24.08 (2.85). Overall, 37 (44.6%) of patients had minimal or no formal education, and 55 (66.3%) were married that the average age of their husbands was 51.72 (9.38) years old.

As shown in Table 2, 61 patients had a history of hysterectomy and most of them (73.5%) did not smoke yet. Regarding pathologic characteristics, invasive ductal carcinoma (IDC) was the most common type with around 86.7% (n = 72) of all patients. Most patients were diagnosed at stage II (70.5%) while only 29.5% of them were diagnosed at stage I. About 59% of patients were Her2 ++ while 36.1% and 44.6% of them were ER + and PR ++ respectively.
Table 3 illustrates the biomarkers before and after radiotherapy. As shown, radiotherapy significantly increased the TIGAR (mean difference (SD) = 0.24 ± 0.76, (p = 0.04)) and HO-1 (mean difference (SD) = 0.26 ± 0.77, (p = 0.003)) genes expression. In patients treated with RT the TAC (mean difference (SD) = −287.31 ± 355.85, (p < 0.001)) and total protein (mean difference (SD) = −0.50 ± 0.08, (p = 0.001)) decreased. Although total bilirubin (mean difference (SD) = 0.08 ± 0.16, (p = 0.001)) and direct bilirubin (mean difference (SD) = 0.7 ± 0.14, (p < 0.001)) increased, no significant change in the serum albumin was found (p = 0.104). Regarding liver enzymes, and as shown in Table 3, ALT (mean difference (SD) = 2.51 (11.34), (p = 0.04) and AST (mean difference (SD) = 2.18 (9.26), (p = 0.03) significantly increased after RT. However, we found no statistically significant change in ALP (p = 0.26). For each patient, changes from baseline in TIGAR, HO-1, and TAC levels using a ‘waterfall plot’ is provided as a supplementary file (Supplementary Figure 1–3).

Accordingly, after RT we found a significant increase in both total and direct bilirubin among patients. At baseline total bilirubin was 1.51 (± 0.17) mg/dl and then it reached a level of 1.60 (± 0.19) mg/dl after radiotherapy (p = 0.001). For direct bilirubin, we found a 0.7 (± 0.14) mg/dl increase after radiotherapy which was statistically significant (p value < 0.001). Furthermore, we found that RT may significantly decrease the mean (± SD) of TP from 5.05 (± 1.14) mg/dl in baseline to 4.13 (± 0.76) mg/dl after treatment with RT (p = 0.001). However, as shown in Table 3 no significant change was observed between the mean (± SD) of Albumin before and after RT (p = 0.104).

As Figure 2 illustrates, the mean (± SD) of TIGAR gene expression was significantly increased after RT, in which the value of this marker was 0.99 (± 0.46) before and it was changed to 1.23 (± 0.46) after RT, which is a statistically significant increase (p value = .004) (Figure 2(A)). Similarly, according to the results of paired sample t-test and as shown in Figure 2(B), we found that RT may significantly increase the mean (± SD) of HO-1 gene expression from 1.00 (± 0.67) in baseline to 1.26 (± 0.05) after treatment with RT (p = 0.003). Regarding TAC, a significant decrease was observed after RT. As such, the mean (± SD) of TAC was 939.84 (± 319.97) μmol/L before prescribing radiotherapy and it reached 625.53 (± 265.74) μmol/L after radiotherapy (p < 0.001) (Figure 2(C)).

Correlations between the study markers are presented in Table 4. As shown, there was a weak significant correlation between TIGAR and HO-1 (r = 0.63, p = 0.05), and also, significant correlations were observed between stage of disease with HO-1 (r = 0.29, p = 0.01) and TIGAR (r = 0.28, p = 0.01). Likewise, we found a positive significant correlation between age with TP. As such, increase in age may upsurge the HO-1 (r = 0.23, p = 0.05) and TIGAR (r = 0.23, p = 0.05) and decline TP levels (r = -0.22, p = 0.05).

Additionally, we performed sensitivity analysis by excluding lobular types of tumors, to find out whether any association with the type of tumor may change our findings. Results, however, remained stable and therefore are not reported (details are not presented here to save limited space).

Overall, the results showed that TIGAR and HO-1 augmented significantly after RT. Total and direct bilirubin

### Table 2. Demographic and pathologic characteristics of 83 breast cancer participants.

| Variable                  | Category    | N   | %   |
|---------------------------|-------------|-----|-----|
| Age (mean ± SD)           | Primary     | 37  | 44.60 |
| Age at first childbirth (mean ± SD) | High school | 30  | 36.10 |
| BMI (kg/m²) (mean ± SD)   | Academic    | 16  | 19.30 |
|                          | Married     | 55  | 66.3 |
|                          | Single      | 28  | 33.7 |
| Hysterectomy Yes          | Yes         | 25  | 30.12 |
|                          | No          | 58  | 69.88 |
| Smoking Yes               | Yes         | 22  | 26.5 |
|                          | No          | 61  | 73.5 |
| Tumor type                | Ductal      | 72  | 86.7 |
|                          | Lobular     | 9   | 10.8 |
|                          | Other       | 2   | 2.4  |
| Stage                     | I           | 23  | 29.5 |
|                          | II          | 55  | 70.5 |
| Lateral                   | Left        | 55  | 66.3 |
|                          | Right       | 24  | 28.9 |
|                          | Bilateral   | 4   | 4.8  |
| HER2                      | Positive    | 49  | 59.0 |
|                          | Negative    | 34  | 41.0 |
| ER                        | Positive    | 30  | 36.1 |
|                          | Negative    | 53  | 63.9 |
| PR PR                    | Positive    | 37  | 44.6 |
|                          | Negative    | 46  | 55.4 |

### Table 3. Comparison of changes between pre- and post-values of bio markers among patients treated with RT.

| Marker                  | Pre radiotherapy Mean (SD) | Post radiotherapy Mean (SD) | Post – pre Mean difference (SD) | Paired sample t test p Valuea |
|-------------------------|----------------------------|-----------------------------|--------------------------------|--------------------------------|
| TIGAR                   | 0.99 (0.46)                | 1.23 (0.46)                 | 0.24 (0.76)                     | .004 |
| HO-1                    | 1.00 (0.67)                | 1.26 (0.05)                 | 0.26 (0.57)                     | .003 |
| TAC (μmol/L)            | 939.84 (319.97)            | 625.53 (265.74)             | −287.31 (−355.85)               | .001 |
| TB (mg/dL)              | 1.51 (0.17)                | 1.60 (0.19)                 | 0.08 (0.16)                     | .001 |
| DB (mg/dL)              | 0.74 (0.17)                | 0.82 (0.19)                 | 0.07 (0.14)                     | .001 |
| TP (g/dl)               | 5.05 (1.14)                | 4.54 (1.20)                 | −0.50 (0.08)                    | .001 |
| Albumin (g/dL)          | 4.18 (0.70)                | 4.13 (0.76)                 | −0.05 (0.30)                    | .10  |
| ALT (U/L)               | 56.16 (11.96)              | 58.68 (12.13)               | 2.51 (11.34)                    | .04  |
| AST (U/L)               | 47.45 (13.23)              | 49.63 (11.62)               | 2.18 (9.26)                     | .03  |
| ALP (U/L)               | 138.20 (19.37)             | 139.95 (17.53)              | 1.77 (14.46)                    | .26  |

### Notes:

- ER: estrogen receptor; HER2: human epidermal growth factor receptor; PR: progesterone receptor.

- Markers in bold show significant changes.

- Changes in markers using paread sample t-test.

- HB: Heme oxygenase gene; TAC: Total antioxidant capacity (μmol/L); DB: Direct bilirubin (mg/dl); TB: Total bilirubin (mg/dl); TP: Total protein (g/dl); and Albumin (g/dl), ALT (U/L), AST (U/L), ALP (U/L).

- *Changes in markers using paired sample t-test.*
increased significantly after RT. On the other hand, TAC levels were significantly declined after RT. Furthermore, TIGAR and HO-1 expression were significantly correlated with the age and the clinical grading of the disease. No significant statistical correlations were shown with education, smoking, lymph node status, tumor size, and expression of estrogen and progesterone receptors (data are not shown).

**Discussion**

Today, the various types of successful treatments have been introduced in order to reduce mortality among breast cancer patients (Visvanathan et al. 2019; Waks and Winer 2019). A meta-analysis study of 10,800 patients showed that RT after lumpectomy reduced breast cancer recurrences by approximately half (from 35.0% to 19.3%) and in mortality rate by one-sixth (from 25.2% to 21.4%) at 10 and 15 years, respectively (O’Halloran et al. 2019). Although RT with a standard dose (50 Gy over 25 fractions) had been historically proven to be effective (Whelan et al. 2010; Haviland et al. 2013) such findings confirm the importance of RT treatment for breast cancer patients (Waza et al. 2018). However, long-term RT treatment can lead to thoughtful side effects and complications among patients (Perez et al. 2017).

The results of our study showed a significant increase in the amount of ROS compounds and a significant decrease in the amount of TAC, in serum patients after RT (Figure 2(C)). Several studies have shown that ROS can be both an initiator and an inhibitor in tumor development and metastasis (ten Kate et al. 2006; Le Gal et al. 2015; Armandis et al. 2018; Wiel et al. 2019). Nevertheless, studies show that TAC, a biomarker in biomedical and nutritional studies, inhibits cancer cell growth (Kusano and Ferrari 2008). Therefore, increase in the ROS and decrease of TAC in serum are signs of damage to the cells. In this study, a significant increase in bilirubin, ALT, and AST levels was observed after treatment of patients with radiation therapy, which is in line with the results of a study by Schuffnegger et. al. (Herman et al. 2015). Although ALT and AST may return to normal a few months after the end of radiation therapy.

The over-expression of HO-1 occurred in PBMCs, an enzyme that catalyzes the degradation of heme. Bilirubin is the main products of HO-1 (Ryter and Tyrrell 2000). Serum bilirubin has anti-oxidative, anti-inflammatory, and immunosuppressive functions in the human body. Numerous studies have provided evidence that mildly elevated serum bilirubin concentrations are associated with a better prognosis in cardiovascular, autoimmune, and oncologic disease (Peng et al. 2017).

Bilirubin might suppress tumor cell proliferation in vitro and in vivo (Ollinger et al. 2007). The increase in bilirubin levels in this study can be due to two reasons: first, bilirubin is one of the most important antioxidants in the body and plays a vital role in counteracting ROS and oxidative damage caused by ionizing radiation and radiation therapy. Indeed, the increase in bilirubin can be considered as a compensatory mechanism against ROS produced due to radiation therapy and by inducing activity or increasing the expression of the HO-1 enzyme. Second, because radiation therapy can damage cancer cells and increase blood cell damage and increase catabolism, this may be due to an increase in bilirubin levels. However, due to the significant

**Figure 2.** Changes in TIGAR, HO and TAC using pared samle t-test. A: TIGAR gene expression, B: Heme oxygenase (HO-1) gene expression, C: Total antioxidant capacity (TAC) (μmol/L). (A) TIGAR significantly (p = .004) increased after RT (pre mean = 0.99 (0.46), psot mean = 1.23 (0.46)). (B) HO significantly (p = .003) increased after RT (pre mean = 1.00 (0.67), psot mean = 1.26 (0.05)). (C) TAC significantly (p < .001) decreased after RT (pre mean = 939.84 (319.97), psot mean = 625.53 (265.74)).
increase in direct bilirubin in this study, there is a possibility of liver damage due to radiation therapy in patients with breast cancer. Therefore, due to the importance of bilirubin and their important role in reducing oxidative stress, further studies are recommended in this regard to reduce the role of bilirubin in radiation therapy and the role of this antioxidant in countering the ROS produced by Make radiation therapy clearer to researchers. By protecting tumor cells from oxidative insults, HO-1 was thought to be a critical protective molecule against ROS (Geng et al. 2018).

In the present study, an increase in HO-1 gene expression was observed in patients after RT. Given the crucial role of HO-1 in the fight against various cancers, especially breast cancer, the increased expression of this gene may be explained by the fact that radiation therapy activates various pathways in the body, some of which are sensitive.

Cancer cells are exposed to ionizing radiation, and there are pathways that compensate for these complications and damage. Therefore, increasing HO-1 levels may be a situation to deal with. However, part of this increase may be due to increased catabolism and increased HO-1 enzyme activity in patients receiving radiation therapy. Studies have shown that the expression of HO-1 in tumor cells after anti-tumor therapy, such as chemotherapy, radiation therapy, photodynamic therapy, increases and protects them from apoptosis induced by these factors, resulting in cancer cell death. The increase in expression of HO-1 through medication has reduced the tumor size in two laboratory animal models (Gandini et al. 2019). Also, Hill et. al. reported an increase in HO-1 through radiation therapy in breast cancer patients (Shi and Fang 2008).

The over-expression of TIGAR was mainly found in some cancer cells that reduces ROS and promote tumor growth (Martinez-Outschoorn et al. 2010) that results in the chemotherapy resistance(Martinez-Outschoorn et al. 2011). Moreover, ROS can act as a dualistic messenger to promote or inhibit the expression of TIGAR. In normal cells, ROS plays the central role in the over-expression of the TIGAR(Saretzki 2010). The over-expression of TIGAR in normal PBMC in patients treated with RT caused the decrease in the serum ROS and increase in the TAC as a compensatory mechanism.

To sum up, the findings of this study indicated that the treatment of patients with RT causes adverse effects on the body, these injuries are compensated by some mechanisms, one of which may increase in the production of bilirubin as the body’s most important antioxidant and the other mechanism may be due to the TIGAR-related pathways that are against ROS and cell damage.

Table 4. Correlation between age, BMI, and stage with mean difference of bio markers.

|                | Stage | Age | HO-1  | TIGAR | TP     | TAC        | Albumin | DB  | TB  |
|----------------|-------|-----|-------|-------|--------|------------|---------|-----|-----|
| **Pearson correlation** |       |     |       |       |        |            |         |     |     |
| Stage          | 1     | 0.084 | 0.286b | 0.282b | −0.024 | −0.166     | −0.060  | 0.044 | 0.023 |
| Age            | 0.084 | 1    | 0.239a | 0.228a | −0.225a | −0.119     | −0.003  | 0.110 | −0.067 |
| TIGAR          | 0.282b | 0.228a | 1      | 0.203  | 0.120  | −0.127     | 0.210   | −0.122 |

HO: Heme oxygenase gene; TAC: Total antioxidant capacity (μmol/L); DB: Direct bilirubin (mg/dl); TB: Total bilirubin (mg/dl); TP: Total protein (g/dl), and Albumin (g/dl).

Conclusion
Due to the antioxidant and peroxidation role of the HO-1 enzyme as well as TIGAR, which protects cells from DNA damage by ROS, the increased levels of these two genes in this study could indicate a compensatory mechanism in maintaining the body’s homeostasis.

It seems that the damage caused by RT, which may cause damage to healthy cells in the body, will be reversed over time by the body’s compensatory and defense mechanisms. Therefore, understanding the ways and mechanisms of compensation, as well as the pathways that are affected by the treatment of patients with radiation therapy can help in better understanding and understanding this treatment and treatment of breast cancer.

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