The Relationship of Ribosomal Protein L10 (RPL10) and L41 (RPL41) with Breast Cancer Molecular Subtypes

Sebati Sinan URKMEZ[1], Birsen BILGICI[1], Yurdanur SULLU[2], Bekir KURU[3], Necati OZEN[4], Leman TOMAK[5], Mehmet Alper ARSLAN[6]

[1] Ondokuz Mayis University, Faculty of Medicine, Department of Medical Biochemistry
[2] Ondokuz Mayis University, Faculty of Medicine, Department of Pathology
[3] Atasam Medical Center, Department of General Surgery
[4] VM Medical Park Hospital, Department of General Surgery
[5] Ondokuz Mayis University, Faculty of Medicine, Department of Biostatistics
[6] Ondokuz Mayis University, Faculty of Medicine, Department of Medical Biology

ABSTRACT

Breast cancer represents the most prevalent cancer type among women globally. Accurate molecular subtyping of breast cancer plays a vital role in determining optimal treatment strategies. Therefore, the main objective of this observational study is to investigate the correlation between breast cancer and its molecular subtypes with ribosomal proteins L10 and L41 at the level of gene expression. A total of 58 cancer patient samples, along with 16 healthy controls, were utilized. The samples were classified into molecular subtypes based on immunohistochemistry analysis. Tissue samples were subjected to RT-qPCR analysis for measurement the gene expression levels of RPL10 and RPL41. The findings revealed no significant differences in RPL10 gene expression across molecular subgroups of breast cancer. However, a significant decrease in RPL41 gene expression by 0.253-fold ($p< 0.05$) in the HER2-rich subtype and 0.257-fold ($p< 0.05$) in the TNBC subtype was observed compared to the control group. Additionally, RPL41 gene expression was significantly downregulated by 0.37-fold ($p< 0.05$) in the whole breast cancer group. In conclusion, the study results indicate a significant downregulation of RPL41 gene expression in the HER2-rich and triple-negative breast cancer (TNBC) breast cancer subtypes, as well as in the overall breast cancer cohort. To better understand the roles of RPL41 and RPL10 in cancer biology, further comprehensive investigations, including functional studies and mechanistic experiments, are needed.

Keywords: Breast cancer, Gene expression, RPL10, RPL41

INTRODUCTION

Breast cancer represents the most prevalent form of malignancy among women, and it stands as the second leading cause of cancer-related mortality following lung cancer.[1] Molecular subtyping of breast cancer plays a pivotal role in guiding the development of integrative treatment strategies, including surgery, radiotherapy, chemotherapy, endocrine therapy, and targeted therapy. Such comprehensive approaches have proven instrumental in enhancing the efficacy of treatment, leading to improved overall survival and progression-free survival rates.[2,3] Breast cancer subtyping is determined based on the expression levels of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor-2 (HER2), and the Ki67 proliferation index.[4] According to the Gallen Consensus of 2013, breast cancer can be classified into four molecular subtypes: Luminal A [ER (+); PR (+/-); HER2 (-); low Ki67], Luminal B [ER (+); PR (+/-); HER2 (+/-); high Ki67], HER2-rich [ER (-); PR (-); HER2 (+)], and TNBC [ER (-); PR (-); HER2 (-)].[5]

The ribosome, a complex macromolecular machinery, comprises ribosomal proteins and ribosomal RNA (rRNA). Ribosomal proteins have crucial functions as RNA chaperones, actively participating in ribosomal particle assembly and stabilizing critical regions of rRNA.[6,7] In addition to their vital role in ribosome stability, ribosomal proteins have demonstrated secondary functions that have not been fully characterized yet. These functions might have a role in diverse cellular processes, including DNA repair, apoptosis, drug resistance, proliferation, and growth inhibition.[8,9]

In cancer cells, there is an elevated requirement for protein synthesis, which necessitates highly efficient ribosomes compared to normal cells. As a matter of fact, several tumor suppressor and oncogenic proteins are known to regulate ribosome biogenesis and overall protein synthesis, thereby exerting control over the progression of cancer cells.[10] Hence, ribosomal proteins have emerged as significant contributors to tumorigenesis. For example, studies have shown that the overexpression of RPS3A leads to neoplastic transformation of the NIH-3T3 cell line and promotes tumor growth in mice.[11] On the other hand, RPS13, which is highly expressed in gastric cancer cells, impedes drug-induced apoptosis and boosts gastric cancer cell proliferation.[12] Intriguingly, ribosomal proteins also exhibit diverse tumor-suppressive effects. Notably, RPL41 has been identified as a crucial factor in restoring sensitivity to chemotherapy in drug-resistant cancer cells.[13] Additionally, ribosomal proteins have been found to suppress tumorigenesis by activating tumor suppressor mechanisms. Notably, three specific ribosomal proteins, namely RPL11, RPL5, and RPL23, when released from the pre-ribosome into the nucleoplasm, directly bind to MDM2, inhibiting MDM2-mediated p53 ubiquitination and degradation, ultimately leading to p53 associated events such as cell cycle arrest, reduced proliferation, and even apoptosis.[14]

Ribosomal protein L10 (RPL10) is a structural protein found in the 60S subunits of ribosomes across various species, ranging from yeast to humans, with an approximate molecular weight of 24 kDa. The gene encoding RPL10 is located in the q28 region on the X chromosome and is alternatively known as QM or DXS648.[15,16] RPL10 indeed plays a crucial role in various cellular processes, including inducing cell proliferation, migration organogenesis and differentiation.[17] Initially, RPL10 complementary DNA (cDNA) was discovered in Wilms tumor (Nephroblastoma).[18] In human epithelial ovarian cancer and prostate cancer elevated RPL10 levels were associated with enhanced cell viability, migration, invasion, and reduced apoptosis.[19-22] Furthermore, a RPL10 mutation R98S was associated with acute T-lymphoblastic leukemia.[23] In non-inflammatory, hormone-negative breast cancers with a poor prognosis and a higher metastasis rate, a study found that RPL10 was linked to reduced metastasis-free survival.[24]

Ribosomal protein L41 (RPL41) is a small peptide composed of 25 amino acids, with a molecular weight of 3.4 kDa. The gene encoding RPL41 is situated in the 12q13.2 region on chromosome 12.[25] In cholangiocarcinoma and nasopharyngeal carcinoma RPL41 gene was seen to be overexpressed.[26,27] However, a study found RPL41 deletion in a significant proportion (59%) of tumor cell lines. Additionally, 75% of primary breast cancers exhibited downregulated RPL41 gene expression.[13]

Although the aforementioned ribosomal proteins have been extensively studied across various cancer types, including breast cancer, they have not been directly correlated with the molecular subtypes of breast cancer. The primary objective of this present study was to evaluate the association between the gene expressions of RPL10 and RPL41 with breast cancer and its molecular subtypes. The secondary aim was to investigate the correlation of RPL10 and RPL41 expression levels with the immunohistochemical markers used in subtyping, and to examine the diagnostic value of RPL10 and RPL41 expression levels. Our study is the first of this type to elucidate the molecular contribution of RPL10 and RPL41 in the development and evolution of breast cancer molecular subtypes.

MATERIALS AND METHODS

Characteristics of the Study Population

The study was designed to include 74 samples across breast cancer group (n= 58) and control group (n= 16). According the result of the immunohistochemical evaluation, the samples were di-

vided into molecular subtypes. Patients with ER and/or PR positive, HER2 negative, Ki67 proliferation index below 14% were classified as luminal A (n= 16); Patients with ER and/or PR positive, HER2 negative or positive, Ki67 proliferation index of 14% and above were classified as luminal B (n= 17); patients with any Ki67 proliferation index, ER, PR negative, HER2 positive were classified as HER2-rich (n= 13); Patients with any Ki67 proliferation index and negative ER, PR, and HER2 were classified as TNBC (n= 12).[4]

Tissue samples consisting of the subjects and control groups were from female volunteers with ages above 18 who signed the consent forms. The samples from the breast cancer groups (n= 58) were obtained routinely during the mastectomy procedure while admitted to Ondokuz Mayıs University Medical Faculty Hospital, Department of General Surgery. The samples of the control group (n= 16) were formed from healthy tissues obtained from volunteers and did not show any neoplastic and inflammatory findings on histopathological examination and belonged to non-cancer surgery procedures.

Immunohistochemical Analysis and Determination of Molecular Subtypes of Specimens

After the surgical procedure, macroscopic examinations were performed on the unfixed tissue samples, which were then sent to the Pathology laboratory for further analysis. For each sample, a piece of tumor tissue was frozen in DNAase and RNAase free conditions for subsequent gene expression analysis by RT-qPCR method and kept at -80^{0}C until the beginning of the study. The remaining tumor tissue samples destined for immunohistochemical studies were fixed in 10% neutral formalin for 24 hours and then processed. Four-micron sections were then taken from the prepared paraffin blocks, stained with hematoxylin-eosin, and examined under a light microscope. Histological typing and grading of tumors were performed.[28] Immunohistochemical evaluations were performed on an automated immunostaining device (Ventana Benchmark XT, Ventana Medical Systems, France and Ventana Benchmark Ultra, Ventana Medical Systems, Tucson, USA) according to the manufacturer's instructions. Anti-estrogen receptor rabbit monoclonal primary antibody (clone SP1, Ventana, USA) for ER, anti-progesterone receptor rabbit monoclonal primary antibody (clone 1E2, Ventana, USA) for PR, anti-HER2 rabbit monoclonal antibody (clone 4B5, Ventana, USA) for HER2, and anti-Ki67 rabbit monoclonal primary antibody (clone 30-9, Ventana, USA) for Ki67, were used. All antibodies were ready to use. The ER, PR, HER2, and Ki67 proliferation index parameters were evaluated for each sample. An ER and PR positivity cutoff of 1% or more was used, where nuclear staining at or above this threshold was considered indicative of positive expression.[29] HER2 expression was evaluated according to the ASCO/CAP 2018 guideline, and complete, intense membranous staining in more than 10% of tumor cells was evaluated as positivity (Score 3). Silver in situ hybridization (SISH) study was performed on suspicious (Score2) cases using a dual SISH probe (INFORM HER2 Dual ISH DNA Probe Cocktail, Ventana). Scores 0 and 1+ were accepted as negative.[30] Ki67 expression was evaluated by counting at least 500 cells in at least three high magnification (X40) fields, including hot spot areas and heterogeneously stained areas. It was scored as the ratio of stained cells to total tumor cells.[31] The samples were divided into four molecular subgroups according to these parameters.[5] In the control group, breast tissues were obtained from patients who were not subjected to cancer surgery. A macroscopic examination was carried out for each control sample in the pathology laboratory. Specimens that did not show any neoplastic signs were included in the control group.

Tissue Homogenization and Gene Expression Analysis

Each tissue sample, weighing thirty milligrams, was pulverized using liquid nitrogen in a pre-cooled mortar and pestle. The pulverization process continued until a fine powder consistency was achieved. The powder was transferred to a new RNAase/DNAase free microcentrifuge tube kept in ice. RNA was extracted from homogenized tissues using the combined TRIzol (Hibrigen, Turkey) method and the FavorPrep™ Tissue Total RNA Mini Kit washing solutions and filters (Favorgen, Taiwan). Quantitative and qualitative evaluations of the isolated RNA were performed

Table 1. Immunohistochemistry profile of cancer samples

	Positive (n; %)	Negative (n; %)	Total (n; %)
Estrogen receptor (ER)	32; 55.2	26; 44.8	58; 100
Progesterone receptor (PR)	30; 51.7	28; 48.3	58; 100
Human epidermal growth factor receptor-2 (HER2)	17; 29.3	41; 70.7	58; 100
	High (n; %)	**Low (n; %)**	
Ki67 proliferation index	42; 72.4	16; 27.6	58; 100

using the Nano-Drop 2000 spectrophotometer (Thermo Scientific, USA). The extracted RNA was QC on agarose electrophoresis under desaturating conditions. cDNA was prepared from 400 ng total RNA using the iScript™ cDNA synthesis kit (Bio-Rad, USA) according to the manufacturer's instructions. qPCR was performed in a CFX96 real-time PCR detection system (Bio-Rad, USA) using the SYBR green (SsoAdvanced™ UNIVERSAL SYBR® Green Supermix, Bio-rad, ABD) method based on the manufacturer's instructions. The amplification of unique products in each reaction was validated by melting curve and agarose gel electrophoresis. The expression level of each gene was normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) expression level using the 2–ΔΔCt method and specific RPL10 and RPL41 primers. The primer sequences used for PCR reactions were as follows: RPL10 (forward) 5′-AGCTGCAGAACAAGGAGCAT-3′ and (reverse) 5′-GTGAAGCCCCACTTCTTTGA-3′; RPL41 (forward) 5′-ATGAGAGCCAAGTGGAGGAA-3′ and (reverse) 5′-TCAGAGGGCGATGAAGTTCT-3′; GAPDH (forward) 5′-TCGGAGTCAACGGATTTGGT-3′ and (reverse) 5′-TTCCCGTTCTCAGCCTTGAC -3′.

This study has been prepared in accordance with the Declaration of Helsinki and was ethically approved by the local Ondokuz Mayıs University, Clinical Research Ethics Committee with the decision numbered OMU KAEK 2020/78.

Statistical Analysis

The Shapiro-Wilk test was used to analyze the assumption of normal distribution of quantitative results. RT-qPCR results were evaluated using the QIAGEN 2009 relative expression software (REST).[32] Logarithmic transformation was applied when creating the main expression graph. Continuous data that did not fit into the normal distribution were evaluated with Spearman rank correlation analysis. For pairwise comparisons, the Mann-Whitney U test was employed, and the data were expressed as median with interquartile range (IQR). ROC analysis was used to determine the diagnostic value of the study data.

RESULTS

The age range of healthy subjects was 29 to 67 years, with an average age of 43.94±8.82 years. For cancer patients, the age range was 32 to 78 years, with an average age of 53.47±10.56 years. Cancer samples were graded according to the Modified Scarff-Bloom-Richardson grading system. Accordingly, 6.9% (n= 4) of the cases were found to be Grade 1; 25.9% (n= 15) were found to be Grade 2; 67.2% (n= 39) were found to be Grade 3. In histological typing, 82.8% (n= 48) of the cases were detected as invasive ductal carcinoma and 17.2% (n= 10) as invasive lobular carcinoma.

According to immunohistochemical analysis of the cancer samples, 32 (55.2%) samples were found ER-positive, 30 (51.7%) samples were found PR positive, and 17 (29.3%) samples were found HER2 positive. Ki67 proliferation index was 14% and above in 42 (72.4%) cases (Table 1).

Some samples were excluded from the study because the Ct signal could not be obtained. Groups according to the received Ct signal were as follows: In the luminal A group, n= 14 for RPL10, n= 13 for RPL41. In the luminal B group, n= 15 for RPL10, n= 15 for RPL41. In the HER2-rich group, n= 13

Table 2. RPL10 and RPL 41 gene expression results (REST analysis)

	Total breast cancer vs healthy control					
	Efficiency	Expression	Standard Error	95% CI	p	Result
GAPDH	1.0	1.000	-	-	-	-
RPL10	1.0	0.895	0.090 - 9.034	0.011 - 65.059	0.824	-
RPL41	1.0	0.37	0.042 - 3.972	0.007 - 28.117	0.022	DOWN
			Luminal A vs healthy control			
GAPDH	1.0	1.000				
RPL10	1.0	0.399	0.030 – 5.258	0.005 – 47.143	0.201	-
RPL41	1.0	0.381	0.032 – 3.374	0.007 – 39.355	0.161	-
			Luminal B vs healthy control			
GAPDH	1.0	1.000	-	-	-	
RPL10	1.0	0.873	0.083 – 9.059	0.013 – 80.145	0.82	-
RPL41	1.0	0.621	0.079 – 5.951	0.015 – 37.798	0.381	-
			HER2 rich vs healthy control			
GAPDH	1.0	1.000	-	-	-	-
RPL10	1.0	1.042	0.115 - 9.055	0.015 - 56.578	0.953	-
RPL41	1.0	0.253	0.029 - 3.323	0.005 - 14.264	0.027	DOWN
			TNBC vs healthy control			
GAPDH	1.0	1.000	-	-	-	-
RPL10	1.0	2.015	0.390 - 12.528	0.129 - 63.983	0.176	-
RPL41	1.0	0.284	0.045 - 3.372	0.008 - 10.230	0.035	DOWN

for RPL10, n= 13 for RPL41. In the TNBC group, n= 12 for RPL10, n= 12 for RPL41. In the control group, n= 13 for RPL10 and n= 14 for RPL41.

Without dividing the study population into molecular subclasses, it was observed that RPL41 gene expression was downregulated 0.37-fold and statistically significant (p= 0.022) when the total breast cancer group was compared to the healthy control group. There was no statistically significant change (p= 0.824) in RPL10 gene expression. There was no significant change in RPL10 and RPL41 gene expression in Luminal A and B groups, respectively, compared to the control group (p= 0.201, p= 0.161 for Luminal A; p= 0.82, p= 0.381 for Luminal B). In the evaluation made in the HER2-rich group, it was observed that the RPL41 gene expression was downregulated by 0.253-fold, which was statistically significant (p= 0.027). In the TNBC group, there was an increased trend in RPL10 gene expression compared to the control group, but this phenomenon was not statistically significant (p= 0.176). There was a statistically significant downregulation of RPL41 gene expression by 0.284-fold (p= 0.035) in the TNBC group compared to the control group (Table 2 and Figure 1).

The gene expression levels of RPL10 and RPL41 were examined in both invasive ductal carcinoma (IDC) and invasive lobular carcinoma (ILC) subtypes of breast cancer. It was found that the expression of RPL10 was elevated in IDC compared to ILC, with a fold change of 1.55 (IQR: 2.96) for IDC and 2.22 (IQR: 2.00) for ILC, although this difference was not found to be statistically significant (p= 0.295). Conversely, the expression of RPL41 was observed to be lower in IDC compared to ILC, with a fold change of 0.40 (IQR: 0.84) for IDC and 0.05 (IQR: 0.39) for ILC, and this difference was also not found to be statistically significant (p= 0.682).

The correlation analysis demonstrated the following significant findings: a weak negative correlation between RPL10 gene expression and progesterone receptor (p< 0.05; r= -0.284); a weak positive correlation between RPL10 gene expression and Ki67 index (p< 0.05; r= 0.276); and a weak positive cor-

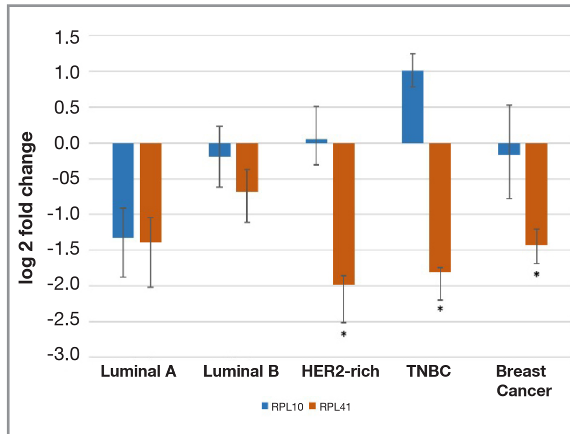

Figure 1. Changes in the gene expression of RPL10 and RPL41 among the molecular subtypes of total breast cancer. The data were presented as log2 transformed. * p< 0.05

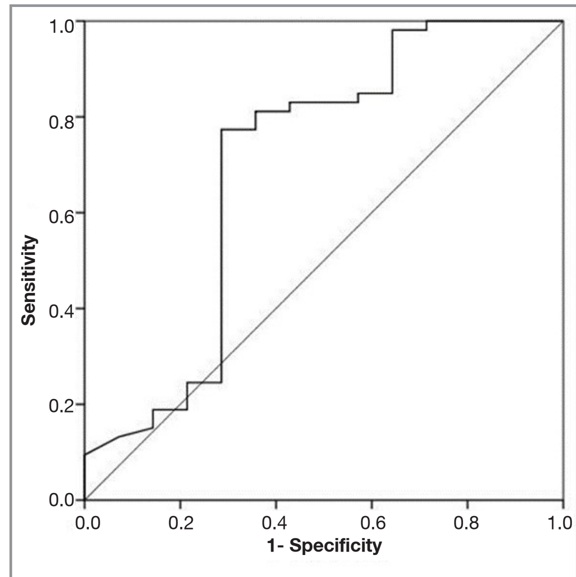

Figure 2. Receiver operating characteristic (ROC) analysis was performed to assess the RPL41 gene expression levels (log10) for the entire group of individuals with breast cancer

relation between the RPL10 and RPL41 parameters ($p < 0.05$; $r = 0.331$). However, no statistically significant correlations were observed between these parameters and other immunohistochemistry parameters (Table 3).

The ROC analysis shows that the RPL41 gene expression level was 0.86 times lower ($p = 0.02$) in the breast cancer group, indicating that this parameter can be used for the diagnostic purpose of breast cancer. When the cutoff value was 0.86, the sensitivity was 77%, the specificity was 71%, and the AUC was 0.697. RPL10 expression was not of diagnostic value in breast cancer and its molecular subtypes (Figure 2).

DISCUSSION

Based on the findings of this study, the significant downregulation of RPL41 gene expression in the HER2-rich and TNBC groups compared to the control group, as well as in the overall breast cancer cohort, suggests a potential association between RPL41 and cancer. In a study focusing on RPL41, malignancy transformation was observed in RPL41 knockout (KO) fibroblast cell lines. Additionally, downregulation of RPL41 was detected in 75% of primary breast cancers, and RPL41 deletions were found in 59% of tumor cell lines in the same study. It has been demonstrated that downregulated RPL41 expression results in abnormal mitosis characterized by frequent cytokinesis failure and increased polynuclear cells, potentially contributing to genome instability and malignant transformation. Although RPL41 was not associated with midbody in proteomic analysis, its localization there was clearly demonstrated in our study. Additionally, RPL41's unique composition may impede its detection in proteomic studies. Furthermore, lagged chromatids were frequently observed in RPL41-depleted cells, possibly due to shortened

Table 3. Correlation of RPL10 and RPL41 gene expressions with immunohistochemistry parameters

		ER	PR	HER2	Ki67	RPL10	RPL41
RPL10	r	-0.174	-0.284*	0.032	0.276*	-	0.331
	p	0.208	0.038	0.819	0.043	-	0.020
RPL41	r	0.228	0.126	-0.204	-0.163	0.331*	-
	p	0.101	0.368	0.143	0.243	0.020	-

mitotic spindles, leading to DNA fragmentation and loss of tumor suppressors. Premature centrosome splitting was observed in these cells, suggesting its potential role in tumorigenesis.[13]

Another study investigating RPL41 demonstrated a decrease in protein levels in retinoblastoma samples.[33] In a study examining human nasopharyngeal carcinoma and primary cell lines derived from healthy nasopharyngeal epithelium, RPL41 gene expression was found to be significantly downregulated in carcinoma cell lines when compared to healthy cell lines.[27] A thorough analysis of these recent findings, when compared with the results of our current study, reveals a correlation between the observed patterns. While these results collectively imply a potential role of RPL41 in cancer pathogenesis, it is important to note that the precise functional impact of RPL41 downregulation in the context of cancer remains to be fully elucidated. The decreased expression of RPL41 in various cancers and this issue associated increase in tumor growth and aggressiveness suggests that RPL41 may exert tumor-suppressive effects during the initiation and/or progression stages of cancer.

Interestingly, based on these results, we hypothesize a plausible connection between the observed downregulation of RPL41 and the previously reported upregulation of a transcription factor ATF4 in HER2-rich and TNBC groups in the literature.[34-39] ATF4 is a member of the ATF/CREB family and belongs to the bZIP transcription factor group. ATF4 forms functional heterodimers with other ATF members and regulates target gene expression by binding to DNA sequences known as cAMP response elements (CRE) or C/EBP-ATF response elements (CARE).[40-42] It serves as a crucial transcription factor in cellular responses such as ER stress and oxidative stress. Within the ER stress signaling pathways, the PERK/eIF2α pathway specifically regulates ATF4 expression.43 ATF4 plays a decisive role in determining whether cells enter a life-saving or apoptotic pathway under ER stress conditions. It contributes to cell survival by regulating the expression of amino acid transporters, metabolic enzymes, and ER chaperones, thus aiding in stress alleviation.[44,45] Additionally, ATF4 expression is upregulated in cancers, supporting proliferation, cell survival, drug resistance, migration, and metastasis.[37,46,47] Indeed, there are numerous studies demonstrating the close relationship between ATF4 and RPL41. In one study, it was demonstrated that RPL41 triggers the translocation of ATF4 from the nucleus to the cytoplasm, accompanied by the presence of a proteasome marker, leading to the rapid degradation of ATF4. Furthermore, this study reported a significant increase in ATF4 expression in cells with RPL41 deletion.[40-48] These findings suggest that RPL41 may have a physiological role in regulating cellular ATF4 levels. A study investigating the effects of recombinant RPL41 administration on retinal neovascularization in a retinopathy experimental animal model has shown that after intravitreal injection of recombinant RPL41, the size of retinal neovascularization and vaso-obliteration were significantly reduced. It has been reported that the gene and protein expression of ATF4 was significantly decreased after recombinant RPL41 injection.[49] Additionally, the administration of recombinant RPL41 in a retinoblastoma cell culture model leads to ATF4 degradation, promoting apoptosis and cell cycle arrest in Y79 and Weri-Rb1 cells.[33] These results highlight the importance of RPL41 as a potential adjuvant in cancer therapy. It has been demonstrated that the administration of low-dose recombinant RPL41 can sensitize tumor cells to the DNA damage agent cisplatin in a lung cancer cell line.[37] Furthermore, low-dose administration of RPL41 resulted in a significant increase in the antitumor effect of carboplatin, a chemotherapeutic agent, and has successfully re-sensitized retinoblastoma cells that were resistant to this agent.[48] These findings suggest that the application of recombinant RPL41 may provide support to conventional treatments in overcoming existing chemotherapy resistance. Taking into account these literature findings and the comprehensive results in our study, targeting the replacement of RPL41 expression, which may be decreased in HER2-rich and triple-negative breast cancer subtypes, could represent a novel treatment approach.

However, it is worth noting that certain studies in the literature have reported contradictory results compared to our obtained data. In one study, RNA-Seq data in seven intrahepatic cholangiocarcinoma tumor and peritumor healthy tissue sam-

ple pairs were analyzed and it was shown that the RPL41 gene was upregulated in tumor tissues.[26] When the RNA sequence data of epithelial ovarian cancer and normal ovarian samples in various databases were analyzed by Differential Gene Expression analysis by another study group, it was reported that RPL41 and ATF4 gene expressions were up-regulated.[50]

The possible reason for this difference may be related to types of cancer. Different types of cancer may exhibit unique genetic changes and molecular pathways, resulting in a variety of gene expression patterns. Additionally, if we assume that RPL41 is anti-cancer, its expression may have increased as a response to the neoplastic effect in these cancer types, particularly during the early stages.

The expression levels of RPL10 have been extensively investigated, and differing results from overexpression to underexpression to the presence of pathogenic mutations have been found in various types of cancer.[17-24] In our study, we found no statistically significant changes in RPL10 gene expression among different molecular subtypes of breast cancer compared to the control group. However, when considering the prognostic aspect, we observed an increasing trend in RPL10 gene expression as the prognosis of the patient groups worsened. Additionally, we found a statistically significant but weak correlation ($p< 0.05$; r: 0.276) between RPL10 gene expression and the Ki67 proliferation index, which serves as a prognostic marker. These findings are consistent with some studies in the literature. For instance, in a study, genetic signature formed by alternative splicing products of RPL10 in hormone-negative and non-inflammatory breast cancers is associated with decreased metastasis-free survival rates.[24] Furthermore, there have been suggestions that RPL10 levels may be related to tumor prognosis in prostate cancer.[19-20] In human epithelial ovarian cancer, high RPL10 levels have been associated with increased cell viability, migration, invasion, and decreased apoptosis.[21-22]

Study limitations include the absence of clarification regarding the effects of alterations in RPL10 and RPL41 gene expression on cancer biology, as substantiated by a protein assay. Additionally, the study is constrained by a relatively small sample size and the absence of in vivo or in vitro loss-of-function or gain-of-function experiments to support the current data.

Conclusion

The results indicate a significant downregulation of RPL41 gene expression in the HER2-rich and TNBC breast cancer subtypes, as well as in the overall breast cancer cohort. This suggests a potential association between RPL41 and breast cancer pathogenesis, especially in these aggressive subtypes. However, it would be premature to definitively conclude that RPL41 has tumor-suppressor effects or that carcinogenesis causes its downregulation solely based on these findings. More comprehensive investigations, including functional studies and mechanistic experiments, are needed to better understand the role of RPL41 and RPL10 in cancer biology. Despite the limitations, RPL 41 represents a potential target for innovative cancer therapies and merits further investigation in future research endeavors.

REFERENCES

1 Jemal A, Bray F, Center MM, et al. Global cancer statistics. CA Cancer J Clin 61: 69-90, 2011.

2 Edenfield J, Schammel C, Collins J, et al. Metaplastic breast cancer: Molecular typing and identification of potential targeted therapies at a single institution. Clin Breast Cancer 17: e1-e10, 2017.

3 Ma F, Guan Y, Yi Z, et al. Assessing tumor heterogeneity using ctDNA to predict and monitor therapeutic response in metastatic breast cancer. Int J Cancer 146: 1359-1368, 2020.

4 Prat A, Pineda E, Adamo B, et al. Clinical implications of the intrinsic molecular subtypes of breast cancer. Breast 24: 26-35, 2015.

5 Goldhirsch A, Gelber RD, Piccart-Gebhart MJ, et al. 2 years versus 1 year of adjuvant trastuzumab for HER2-positive breast cancer (HERA): an open-label, randomised controlled trial. Lancet 382: 1021-1028, 2013.

6 Fatica A, Tollervey D. Making ribosomes. Curr Opin Cell Biol 14: 313-318, 2002.

7 Tschochner H, Hurt E. Pre-ribosomes on the road from the nucleolus to the cytoplasm. Trends Cell Biol 13: 255-263, 2003.

8 Shen DW, Liang XJ, Suzuki T, Gottesman MM. Identification by functional cloning from a retroviral cDNA library of cDNAs for ribosomal protein L36 and the 10-kDa heat shock protein that confer cisplatin resistance. Mol Pharmacol 69:1383-1388, 2006.

9 Du J, Shi Y, Pan Y, et al. Regulation of multidrug resistance by ribosomal protein l6 in gastric cancer cells. Cancer Biol Ther 4: 242-247, 2005.

10 Silvera D, Formenti SC, Schneider RJ. Translational control in cancer. Nature reviews. Cancer, 10(4): 254–266i 2010.

11 Naora H, Takai I, Adachi M, Naora, H. Altered cellular responses by varying expression of a ribosomal protein gene: sequential coordination of enhancement and suppression of ribosomal protein S3a gene expression induces apoptosis. J Cell Biol 141(3): 741-753, 1998.

12 Guo X, Shi Y, Gou Y, et al. Human ribosomal protein S13 promotes gastric cancer growth through down-regulating p27(Kip1). J Cell Mol Med 15: 296-306, 2011.

13 Wang S, Huang J, He J, et al. RPL41, a small ribosomal peptide deregulated in tumors, is essential for mitosis and centrosome integrity. Neoplasia 12: 284-293, 2010.

14 Zhou X, Liao JM, Liao WJ, Lu H. Scission of the p53-MDM2 Loop by Ribosomal Proteins. Genes Cancer 3: 298-310, 2012.

15 Farmer AA, Loftus TM, Mills AA, et al. Extreme evolutionary conservation of QM, a novel c-Jun associated transcription factor. Hum Mol Genet 3: 723-728, 1994.

16 Eisinger DP, Dick FA, Trumpower BL. Qsr1p, a 60S ribosomal subunit protein, is required for joining of 40S and 60S subunits. Mol Cell Biol 17: 5136-5145, 1997.

17 Oh HS, Kwon H, Sun SK, Yang CH. QM, a putative tumor suppressor, regulates proto-oncogene c-yes. J Biol Chem 277: 36489-36498, 2002.

18 Dowdy SF, Lai KM, Weissman BE, et al. The isolation and characterization of a novel cDNA demonstrating an altered mRNA level in nontumorigenic Wilms' microcell hybrid cells. Nucleic Acids Res 19: 5763-5769, 1991.

19 Shi J, Zhang L, Zhou D, et al. Biological Function of Ribosomal Protein L10 on Cell Behavior in Human Epithelial Ovarian Cancer. J Cancer 9: 745-756, 2018.

20 Shen XJ, Ali-Fehmi R, Weng CR, et al. Loss of heterozygosity and microsatellite instability at the Xq28 and the A/G heterozygosity of the QM gene are associated with ovarian cancer. Cancer Biol Ther 5: 523-528, 2006.

21 Altinok G, Powell IJ, Che M, et al. Reduction of QM protein expression correlates with tumor grade in prostatic adenocarcinoma. Prostate Cancer Prostatic Dis 9: 77-82, 2006.

22 Albujja MH, Messaudi SA, Vasudevan R, et al. Identification of potential genes for benign prostatic hyperplasia and prostate cancer susceptibility in four X-chromosome regions with high frequency of microvariant alleles. Asian Pac J Cancer Prev 21: 2271–2280, 2020.

23 Girardi T, Vereecke S, Sulima SO, et al. The T-cell leukemia-associated ribosomal RPL10 R98S mutation enhances JAK-STAT signaling. Leukemia 32: 809-819, 2018.

24 Lerebours F, Vacher S, Guinebretiere JM, et al. Hemoglobin overexpression and splice signature as new features of inflammatory breast cancer? J Adv Res 28: 77-85, 2020.

25 Odintsova TI, Müller EC, Ivanov AV, et al. Characterization and analysis of posttranslational modifications of the human large cytoplasmic ribosomal subunit proteins by mass spectrometry and Edman sequencing. J Protein Chem 22: 249-258, 2003.

26 Yang G, Zong H. Overexpression of PDZK1IP1, EEF1A2 and RPL41 genes in intrahepatic cholangiocarcinoma. Mol Med Rep 13: 4786-4790, 2016.

27 Sim EU, Chan SL, Ng KL, Lee CW, Narayanan K. Human ribosomal proteins RPeL27, RPeL43, and RPeL41 Are Upregulated in nasopharyngeal carcinoma cell lines. Dis Markers 2016: 5179594, 2016.

28 Tan PH, Ellis I, Allison K, Brogi E, et al. The 2019 World Health Organization classification of tumours of the breast. Histopathology 77: 181-185, 2020.

29 Allison KH, Hammond MEH, Dowsett M, et al. Estrogen and Progesterone Receptor Testing in Breast Cancer: ASCO/CAP Guideline Update. J Clin Oncol 38: 1346-1366, 2020.

30 Wolff AC, Hammond MEH, Allison KH, et al. Human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Focused Update. J Clin Oncol 36: 2105-2122, 2018.

31 Dowsett M, Nielsen TO, A'Hern R, et al. Assessment of Ki67 in breast cancer: recommendations from the International Ki67 in Breast Cancer working group. J Natl Cancer Inst 103: 1656-1664, 2011.

32 Pfaffl MW, Horgan GW, Dempfle L. Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30: e36, 2002.

33 Geng W, Ren J, Shi H, et al. RPL41 sensitizes retinoblastoma cells to chemotherapeutic drugs via ATF4 degradation. J Cell Physiol 236: 2214-2225, 2021.

34 Zeng P, Sun S, Li R, et al. HER2 Upregulates ATF4 to promote cell migration via activation of ZEB1 and downregulation of E-Cadherin. Int J Mol Sci 20: 2223, 2019.

35 Darini C, Ghaddar N, Chabot C, et al. An integrated stress response via PKR suppresses HER2+ cancers and improves trastuzumab therapy. Nat Commun 10: 2139, 2019.

36 Nam S, Chang HR, Jung HR, et al. A pathway-based approach for identifying biomarkers of tumor progression to trastuzumab-resistant breast cancer. Cancer Lett 356: 880-890, 2015.

37 Singleton DC, Harris AL. Targeting the ATF4 pathway in cancer therapy. Expert Opin Ther Targets 16: 1189-1202, 2012.

38 van Geldermalsen M, Wang Q, Nagarajah R, et al. ASCT2/SLC1A5 controls glutamine uptake and tumour growth in triple-negative basal-like breast cancer. Oncogene 35: 3201-8, 2016.

39 González-González A, Muñoz-Muela E, Marchal JA, et al. Activating transcription factor 4 modulates TGFβ-Induced aggressiveness in triple-negative breast cancer via SMAD2/3/4 and mTORC2 signaling. Clin Cancer Res 24: 5697-5709, 2018.

40 Huggins CJ, Mayekar MK, Martin N, et al. C/EBPγ Is a Critical Regulator of Cellular Stress Response Networks through Heterodimerization with ATF4. Mol Cell Biol 36: 693-713, 2015.

41 Hai T, Curran T. Cross-family dimerization of transcription factors Fos/Jun and ATF/CREB alters DNA binding specificity. Proc Natl Acad Sci U S A. 1: 3720-3724, 1991.

42 Vallejo M, Ron D, Miller CP, Habener JF. C/ATF, a member of the activating transcription factor family of DNA-binding proteins, dimerizes with CAAT/enhancer-binding proteins and directs their binding to cAMP response elements. Proc Natl Acad Sci U S A. 15: 4679-4683, 1993.

43 Wang M, Kaufman RJ. The impact of the endoplasmic reticulum protein-folding environment on cancer development. Nat Rev Cancer 14: 581-597, 2014.

44 Harding HP, Zhang Y, Zeng H, Novoa I, et al. An integrated stress response regulates amino acid metabolism and resistance to oxidative stress. Mol Cell 11: 619-633, 2003.

45 Novoa I, Zhang Y, Zeng H, et al. Stress-induced gene expression requires programmed recovery from translational repression. EMBO J 22: 1180-7, 2003. Erratum in: EMBO J 22: 2307, 2003.

46 Lau E, Feng Y, Claps G, et al. The transcription factor ATF2 promotes melanoma metastasis by suppressing protein fucosylation. Sci Signal 8: ra124, 2015.

47 Yin X, Wolford CC, Chang YS, et al. ATF3, an adaptive-response gene, enhances TGF{beta} signaling and cancer-initiating cell features in breast cancer cells. J Cell Sci 123: 3558-3565, 2010.

48 Wang A, Xu S, Zhang X, et al. Ribosomal protein RPL41 induces rapid degradation of ATF4, a transcription factor critical for tumour cell survival in stress. J Pathol 225: 285-292, 2011.

49 Geng W, Qin F, Ren J, et al. Mini-peptide RPL41 attenuated retinal neovascularization by inducing degradation of ATF4 in oxygen-induced retinopathy mice. Exp Cell Res 369: 243-250, 2018.

50 Siavoshi A, Taghizadeh M, Dookhe E, Piran M. Gene expression profiles and pathway enrichment analysis to identification of differentially expressed gene and signaling pathways in epithelial ovarian cancer based on high-throughput RNA-seq data. Genomics 114: 161-170, 2022.

Correspondence:
Sebati Sinan ÜRKMEZ
Ondokuz Mayis Universitesi
Tibbi Biyokimya Anabilim Dali
55270, Atakum
SAMSUN / TURKIYE
Tel: (+90-554) 614 54 99
e-mail: s.sinanurkmez@gmail.com

ORCIDs:

Sebati Sinan Urkmez	0000-0002-8821-1835
Birsen Bilgici	0000-0001-7783-5039
Yurdanur Sullu	0000-0002-8029-2490
Bekir Kuru	0000-0001-7774-6431
Necati Ozen	0000-0002-8072-9234
Leman Tomak	0000-0002-4138-4472
Mehmet Alper Arslan	0000-0002-3113-7195