

# DEREGULATED EXPRESSIONS OF MYC ALTER THE EXPRESSIONS OF tRNAs IN BREAST CANCER CELLS

## MYC MEME KANSERİ HÜCRELERİNDE tRNA'LARIN İFADESİNİ REGÜLE EDER

Pervin Elvan TOKGÜN<sup>1</sup> , Onur TOKGÜN<sup>1</sup> 

<sup>1</sup>Pamukkale University, Faculty of Medicine, Department of Medical Genetics, Denizli, Türkiye

ORCID ID: P.E.T. 0000-0001 9025-4140; O.T. 0000-0003-0537-9032

**Citation/Atf:** Tokgun PE, Tokgun O. Deregulated expressions of MYC alter the expressions of tnas in breast cancer cells. Journal of Advanced Research in Health Sciences 2023;6(1):88-95. <https://doi.org/10.26650/JARHS2023-1199491>

### ABSTRACT

**Objective:** The protein synthesis process is started with DNA being transcribed into mRNA in the nucleus, then mRNA is transported to the cytoplasm and attaches to a ribosome in order to be translated into a protein. Protein synthesis not only occurs in the cytoplasm. Mitochondria by itself also has its own genetic system. Therefore mtDNA can be replicated, transcribed, and translated. *MYC* oncogene drives the generation of many cancer types. *MYC* also controls some target genes related to ribosome biogenesis and protein synthesis. Increased expressions of tRNA synthetases have been found to be important players in promoting tumor growth in various types of human cancers. In this study we aimed to identify deregulated expressions of tRNAs that are players both in the nucleus and mitochondria in a *MYC*-dependent manner in breast cancer cells.

**Material and Methods:** Cells were infected with lentiviral *MYC* shRNA/overexpression vectors in order to manipulate *MYC* expressions. TRIZOL reagent was used for RNA isolation and libraries were generated for sequencing. The quality of the RNA samples and libraries was assessed using Agilent BioAnalyzer. Sequencing was performed on the Illumina HT2500 platform.

**Results:** We obtained 6 nuclear-encoded mitochondrial tRNAs and 65 nuclear tRNAs as a result of deregulated expression of *MYC*.

**Conclusion:** This study reveals *MYC*-dependent regulation of tRNAs in breast cancer. Further functional studies are required for underlying molecular mechanisms.

**Keywords:** tRNA, next-generation sequencing, breast cancer

### ÖZ

**Amaç:** Protein sentezi süreci, DNA'nın çekirdekte mRNA'ya kopyalanmasıyla başlar, ardından mRNA, bir proteine çevrilmek üzere sitoplazmaya taşınır ve ribozoma bağlanır. Protein sentezi sadece sitoplazmada gerçekleşmez. Mitokondri de kendi genetik sistemine sahiptir. Bu nedenle mtDNA'da replikasyon, transkripsiyon ve translasyon gerçekleşir. *MYC* onkogeni, birçok kanser türünün gelişimine neden olur. *MYC* ayrıca ribozom biyogenezini ve protein sentezi ile ilgili bazı hedef genleri kontrol eder. tRNA sentetazlarının artan ifadelerinin, çeşitli insan kanseri türlerinde tümör büyümesini desteklemede önemli rolü olduğu bulunmuştur. Bu çalışmada, nükleus ve mitokondride görev yapan tRNA'ların *MYC*'e bağımlı ifadelerini belirlemeyi amaçladık.

**Gereç ve Yöntem:** Hücreler lentiviral *MYC* overekspresyon/shRNA vektörleri ile infekte edildi. RNA izolasyonu için TRIZOL reaktifi kullanıldı ve yeni nesil dizileme için kütüphaneler hazırlandı. RNA örneklerinin ve kütüphanelerin kalitesi, Agilent BioAnalyzer kullanılarak değerlendirildi. Dizileme, Illumina HT2500 platformunda yapıldı.

**Bulgular:** Meme kanseri hücrelerinde *MYC* ifade değişimine bağlı olarak 65 nükleer tRNA ve 6 nükleer kodlanmış mitokondriyal tRNA saptandı.

**Sonuç:** Bu çalışma, meme kanserinde tRNA'ların *MYC*'e bağımlı regülasyonunu ortaya koymaktadır. Moleküler mekanizmaların aydınlatılması için daha ileri fonksiyonel çalışmalar gereklidir.

**Anahtar Kelimeler:** tRNA, yeni nesil dizileme, meme kanseri

**Corresponding Author/Sorumlu Yazar:** Pervin Elvan TOKGÜN E-mail: [parslan@pau.edu.tr](mailto:parslan@pau.edu.tr)

**Submitted/Başvuru:** 04.11.2022 • **Revision Requested/Revizyon Talebi:** 04.01.2023 • **Last Revision Received/Son Revizyon:** 04.01.2023

• **Accepted/Kabul:** 04.01.2023 • **Published Online/Online Yayın:** 14.02.2023



This work is licensed under Creative Commons Attribution-NonCommercial 4.0 International License

## INTRODUCTION

The protein synthesis process is started with DNA being transcribed into mRNA in the nucleus, then mRNA is transported to the cytoplasm and attaches to a ribosome in order to be translated into a protein. In this journey, the ribosome reads the chain of codons in the mRNA strand and tRNA translates each three-base codon on the mRNA into an amino acid which creates a polypeptide, that can be processed into a protein. Transfer RNA is an important player in protein synthesis and besides, they also have important roles in the epigenetic regulation of gene expression in various cancers (1).

Protein synthesis not only occurs in the cytoplasm. Mitochondria by itself also has its own genetic system. Therefore mtDNA can be replicated, transcribed, and translated. The double-stranded and circular mitochondrial genome is within a size of 16.5 kb and contains 37 genes encoding 13 proteins, 2 rRNAs and 22 tRNAs (2). The localization of tRNAs are on both the heavy and light strands of the mitochondrial genome and they are transcribed as long polycistronic precursors (3). The tRNA punctuation model was described in 1981 and this model reveals that coding sequences are generally separated by few nucleotides and long polycistronic precursors are processed into mature mRNA and rRNA by cleavage of the 5' and 3'-ends of the flanking tRNAs. (4). tRNAs participate in the protein translation process that mRNAs are converted into an amino acid chain. In mitochondrial translation, the primary processing occurs through cleavage at the 5' end of tRNA by mitochondrial RNase P4 and 3' end by the mitochondrial RNase Z (5,6). mt-RNase P is composed of mitochondrial RNase P proteins 1,2,3 and MRPP1-3 (7). There are four steps of mitochondrial protein translation including initiation, elongation, termination, and ribosome recycling. Mitochondrial translation mechanisms are much more similar to prokaryotic translation (8). For the mitochondrial protein synthesis, MTIF2, MTIF3 (initiation factors), EFTU, EF-TS, mtEF-G1 (elongation factors), MTRF1L (a release factor), MRRF and EF-G2mt (ribosome recycling factors) are required. A deficiency of protein translation factors, mt-tRNAs, mitochondrial ribosome proteins, etc results in abnormal mitochondrial translation, which is associated with a variety of diseases, such as cancer, nervous system diseases, and cardiovascular diseases (9-13).

The structural changes of mtDNA in encoded protein subunits unshared electron transport function and ROS production increases. Carcinogenesis occurs depending on enhanced oxidative stress related to an increased ROS production in mitochondria (14). Increased expressions of tRNA synthetases have been found to be important players in promoting tumor growth in various types of human cancers (15,16). Many studies suggested the important roles of tRNAs in cancer as potential biomarkers.

*MYC* oncogene drives the generation of many cancer types including breast cancer (17-19). *MYC* amplification and overexpression in breast cancer are related to poor outcomes for the patients (20). *MYC* also controls some target genes that

have roles in ribosome biogenesis and protein synthesis (21). After *MYC* binding sites are identified, transcriptional targets are explored and 400 nuclear genes encoding mitochondrial proteins were found to be targets of *MYC* by chromosomal immunoprecipitation analysis of promoter microarrays including genes related to protein import, mitochondrial ribosome, mitochondrial transcription/translation and complex assembly, whereas 198 genes were induced as a result of ectopic expression of *MYC* (22,23). Besides, *MYC* was found to regulate mitochondrial gene expression indirectly via microRNAs (24).

It has been shown that the regulation of tRNA ligases by *MYC* is imputed as a fundamental contributor to *MYC*-driven cell growth in *Drosophila* (25). Even small RNA sequencing performs successful mapping and alignment of gene transcripts and small RNA types including miRNAs, snRNAs, rRNA, and tRNAs, most workflows have been developed for miRNA analysis. But pipelines for the analysis of transfer RNAs are rare. Besides, the roles of tRNAs on cancer development are not very well known. Both mRNAs and tRNAs are important in protein synthesis. Therefore, in the direction of the knowledge that *MYC* regulates both nuclear and mitochondrial genes, here we aimed to illustrate in breast cancer cells if deregulated expressions of *MYC* also affect the expression levels of tRNAs that are players both in the nucleus and mitochondria.

## MATERIALS and METHODS

### Cell culture

MDA-MB-231 cells are cultured in RPMI1640 Medium (GIBCO, USA) containing heat-inactivated 5% FBS (GIBCO, USA), 1% Penicillin-Streptomycin (GIBCO, USA) and 1% L-glutamine (GIBCO, USA) and MCF-7 cells are cultured in DMEM Medium (SIGMA, USA) containing heat-inactivated 5% FBS (GIBCO, USA), 1% Penicillin-Streptomycin (GIBCO, USA) and 1% L-glutamine (GIBCO, USA) and are incubated at 37°C, with 5% CO<sub>2</sub> and 95% humidity in air.

### Lentivirus infection

Inducible lentiviral overexpression/shRNA vectors were designed by Dr.Onur Tokgun (26). MDA-MB-231 and MCF-7 cells were counted and seeded with 3ml medium. Twenty four hours after, cells were infected with a combination of 20 µl lentivirus and 8 µl Polybrene (Sigma Aldrich). After removing the cell media, cells were washed with PBS (GIBCO) twice and replaced with the RPMI140 medium supplemented with 10% Tet-Free FBS (Clonetech) 24 hours after infection. Cells were selected with puromycin in a concentration of 2 µg/ml and induced with Doxycycline (DOX) in a concentration of 2.0 µg/ml.

### RNA isolation

Total RNA was isolated from MCF-7 DX-/DX+, MDA-MB-231 DX-/DX+ cells in a density of 5x10<sup>7</sup> cells grown on T175 flasks. Cell pellets were lysed in 500 µl TRIzol reagent. Subsequently, 100 µl of chloroform was used for phase separation, 100% isopropanol for RNA precipitation and ice-cold 75% ethanol for washing. RNA was eluted in 30 µl RNase-free water. The RNA integrity and purity were measured with a NanoDrop 2000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

### qRT-PCR

Total RNA was reverse transcribed using a High-capacity cDNA reverse transcription kit (Thermo Scientific, Waltham, MA, USA). qRT-PCR reactions were prepared using BioRAD iTaQ Universal SYBR Green Master mix. Oligonucleotide sequences are as follows: *MYC*, F: CTTCTCTCCGCTCGGATTCT R: GAAGGTGATC-CAGACTCTGACCTT; *GAPDH*, F: AGGTCGGTGTGAACGGATTG R: GGGGTCGTTGATGGCAACA. *GAPDH* was used as an endogenous control. mRNA expression of *Myc* between DX+ versus DX- was measured using Eq. 2- $\Delta\Delta C_t$ .

### Small RNA sequencing

Small RNA sequencing was performed at the MacroGen company (South Korea) using the Illumina HT2500 platform. The RNA yield and size distribution of the libraries were analyzed with an Agilent 2100 Bioanalyzer. Libraries generated from 50 ng/ $\mu$ L total RNA samples using the SMART-seq v4 (Takara, America). First, poly(A) tail is added to input RNA using Poly(A) Polymerase in order to facilitate oligo(dT)-primed cDNA synthesis. Then adapter-linked RNA fragments are transformed into cDNA fragments and followed by PCR purification. The sizes of the amplified cDNA fragments were checked using a Bioanalyzer DNA High sensitivity chip. The cDNA fragments are sequenced according to the read length using the "sequence by synthesis" method on the Illumina platform.

### Bioinformatic analysis

Raw sequence readings were filtered and the adapter sequences were removed from the raw sequence readings using the CutAdapt program. The clipped reads are clustered for matching 100% of the sequence and read length. Clustered reads were then aligned with the reference genome (hg19). To classify sRNA types, clustered reads were mapped to the reference genome and non-coding RNA database Rfam v9.1. The expression levels of RNA transcripts in high throughput data are calculated as FPKM (fragments per kilobase of transcript per million reads mapped) (27).

### Statistical analysis

The graphs, calculations, and statistical analyses were performed using GraphPad Prism software version 8.0.1 (GraphPad Software, San Diego, CA, USA). Unpaired Student's t-test was used for comparisons of differential expressions of *MYC*. Statistical results with \*\*\*\* $p < 0.0001$  were considered as statistically significant.

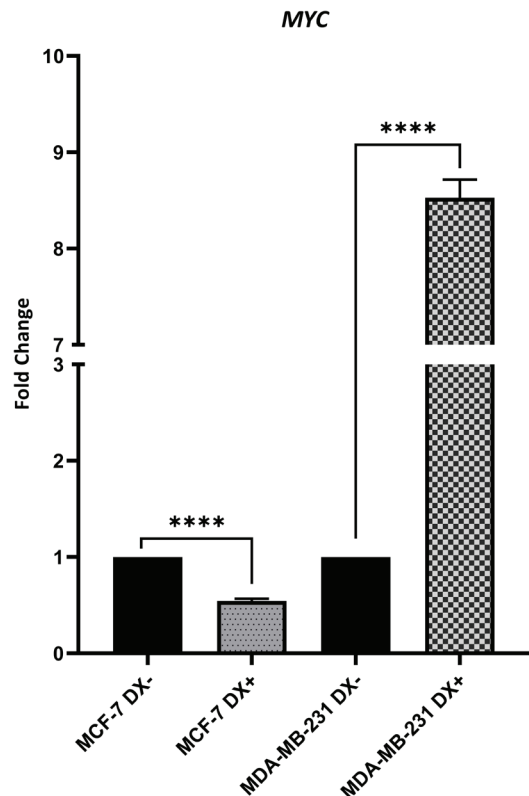
### RESULTS

The deregulated expression levels of *MYC* in breast cancer cells are shown in Figure 1. The total base number, number of readings, GC percentage, Q20 (%), and Q30 (%) were calculated and 3' adapter sequences were removed using the "Cutadapt" program. Sequences smaller than 15 nucleotides were filtered and the reads were matched to the reference genome (hg19), respectively (Table 1). The length analysis of sRNA population was categorized by performing a length analysis.

Rfam v9.1 and Genbank databases were used to determine sRNAs as well as tRNAs. When sRNA sequences are paired with RNAs from Genbank and Rfam databases, tRNAs (MCF-7 DX- 2.71%, MCF-7 DX+ 1.53%, MDA-MB-231 DX- 1% and MDA-MB-231 DX+ 1.66%) was one of the most common sRNA classes in cells. In total, 71 tRNAs were determined, of which 6 of them were nuclear-encoded mitochondrial tRNAs (Table 2). A HeatMap analysis of detected tRNAs are shown in Figure 2.

**Table 1:** Raw Data Statistics and Filtered Number of Reads

| Sample  | Total Reads | GC (%) | Q20 (%) | Q30 (%) |
|---|-------------|--------|---------|---------|
| MDA-MB-231 DX-                                    | 26.377.797  | 43.03  | 84.74   | 79.81   |
| MDA-MB-231 DX+                                    | 32.880.597  | 42.28  | 82.79   | 77.62   |
| MCF-7 DX-   | 34.892.493  | 39.42  | 86.16   | 81.82   |
| MCF-7 DX+   | 45.257.303  | 36.98  | 92.47   | 89.11   |
| <b>Filtered short reads of less than 15 bases</b> |             |        |         |         |
| MDA-MB-231 DX-                                    | 12.245.859  | 68.76  | 97.03   | 93.51   |
| MDA-MB-231 DX+                                    | 14.806.966  | 74.96  | 97.67   | 94.72   |
| MCF-7 DX-   | 13.832.085  | 78.66  | 97.42   | 94.33   |
| MCF-7 DX+   | 14.696.527  | 63.74  | 97.79   | 95.08   |



**Figure 1:** Expression levels of *MYC* after *MYC* inhibition/over-expression in breast cancer cells by lentiviral vectors (\*\*\*\* $p < 0.0001$ ).

**Table 2:** Deregulated expressions of tRNAs depending on *MYC* manipulation

| Gene_Name            | tRNA_Type | Position                    | MDA-MB-231<br>DX- | MDA-MB-231<br>DX+ | MCF-7 DX- | MCF-7 DX+ |
|----------------------|-----------|-----------------------------|-------------------|-------------------|-----------|-----------|
| nmt-tRNA-Gln-CTG-1-1 | Gln-CTG   | chr10:20036618-20036674:-   | 0                 | 3                 | 319       | 7         |
| nmt-tRNA-Gln-TTG-7-1 | Gln-TTG   | chr2:131029911-131029982:-  | 81                | 113               | 2964      | 542       |
| nmt-tRNA-Gln-TTG-9-1 | Gln-TTG   | chr2:132143133-132143204:+  | 86                | 131               | 3206      | 628       |
| nmt-tRNA-Leu-TAA-4-1 | Leu-TAA   | chrX:55207755-55207829:-    | 1498              | 325               | 91        | 171       |
| nmt-tRNA-Ser-TGA-1-1 | Ser-TGA   | chr2:131033024-131033093:-  | 73                | 121               | 957       | 240       |
| nmt-tRNA-Ser-TGA-3-1 | Ser-TGA   | chr17:22028179-22028247:-   | 16                | 56                | 743       | 108       |
| tRNA-Ala-AGC-10-1    | Ala-AGC   | chr6:26687485-26687557:+    | 1                 | 48                | 20        | 3         |
| tRNA-Ala-AGC-4-1     | Ala-AGC   | chr6:28626014-28626085:-    | 1                 | 411               | 98        | 32        |
| tRNA-Ala-CGC-1-1     | Ala-CGC   | chr6:26553731-26553802:+    | 1                 | 406               | 98        | 22        |
| tRNA-Ala-CGC-2-1     | Ala-CGC   | chr6:28641613-28641684:-    | 1                 | 393               | 61        | 23        |
| tRNA-Ala-CGC-3-1     | Ala-CGC   | chr2:157257281-157257352:+  | 1                 | 425               | 72        | 56        |
| tRNA-Ala-TGC-3-2     | Ala-TGC   | chr12:125406301-125406372:- | 1                 | 392               | 72        | 23        |
| tRNA-Ala-TGC-4-1     | Ala-TGC   | chr12:125424512-125424583:+ | 1                 | 407               | 115       | 65        |
| tRNA-Arg-CCG-1-1     | Arg-CCG   | chr6:28710729-28710801:-    | 1                 | 32                | 121       | 49        |
| tRNA-Arg-CCG-2-1     | Arg-CCG   | chr17:66016013-66016085:-   | 1                 | 882               | 468       | 177       |
| tRNA-Arg-CCT-1-1     | Arg-CCT   | chr17:73030001-73030073:+   | 1                 | 372               | 85        | 38        |
| tRNA-Arg-CCT-4-1     | Arg-CCT   | chr7:139025446-139025518:+  | 1                 | 380               | 249       | 38        |
| tRNA-Arg-TCG-3-1     | Arg-TCG   | chr17:73031208-73031280:+   | 1                 | 31                | 125       | 53        |
| tRNA-Arg-TCT-5-1     | Arg-TCT   | chr6:27529963-27530049:+    | 1                 | 28                | 151       | 33        |
| tRNA-Asp-GTC-1-1     | Asp-GTC   | chr12:98897281-98897352:+   | 34                | 1098              | 2804      | 1508      |
| tRNA-Asp-GTC-2-1     | Asp-GTC   | chr1:161410615-161410686:-  | 83                | 1314              | 3090      | 2264      |
| tRNA-Asp-GTC-3-1     | Asp-GTC   | chr6:27551236-27551307:-    | 83                | 981               | 3021      | 1462      |
| tRNA-Asp-GTC-4-1     | Asp-GTC   | chr9:77517990-77518061:-    | 81                | 947               | 2730      | 1356      |
| tRNA-Glu-CTC-1-1     | Glu-CTC   | chr1:145399233-145399304:-  | 8                 | 1797              | 2363      | 1263      |
| tRNA-Glu-CTC-3-1     | Glu-CTC   | chr13:42030061-42030132:-   | 1                 | 577               | 223       | 163       |
| tRNA-Glu-TTC-1-1     | Glu-TTC   | chr2:131094701-131094772:-  | 6                 | 346               | 810       | 240       |
| tRNA-Glu-TTC-2-1     | Glu-TTC   | chr13:45492062-45492133:-   | 7                 | 363               | 873       | 278       |
| tRNA-Glu-TTC-3-1     | Glu-TTC   | chr1:17199078-17199149:+    | 7                 | 1243              | 2063      | 684       |
| tRNA-Glu-TTC-4-1     | Glu-TTC   | chr1:16861774-16861845:-    | 7                 | 1272              | 2179      | 710       |
| tRNA-Gly-CCC-1-1     | Gly-CCC   | chr1:16872434-16872504:-    | 53                | 449               | 200       | 133       |
| tRNA-Gly-CCC-5-1     | Gly-CCC   | chr1:17053780-17053850:+    | 6                 | 309               | 109       | 64        |
| tRNA-Gly-CCC-6-1     | Gly-CCC   | chr1:149680215-149680275:-  | 53                | 380               | 161       | 98        |
| tRNA-Gly-GCC-1-1     | Gly-GCC   | chr1:161413094-161413164:+  | 59                | 613               | 1028      | 270       |
| tRNA-Gly-GCC-2-1     | Gly-GCC   | chr1:161493637-161493707:-  | 59                | 625               | 1058      | 270       |
| tRNA-His-GTG-1-1     | His-GTG   | chr1:145396881-145396952:-  | 1                 | 101               | 226       | 300       |
| tRNA-Ile-AAT-1-1     | Ile-AAT   | chr6:58149254-58149327:+    | 1                 | 116               | 787       | 115       |
| tRNA-Ile-AAT-2-1     | Ile-AAT   | chr6:27655967-27656040:+    | 1                 | 65                | 385       | 57        |
| tRNA-Ile-AAT-5-1     | Ile-AAT   | chr6:26554350-26554423:+    | 1                 | 118               | 787       | 115       |
| tRNA-Ile-AAT-6-1     | Ile-AAT   | chr6:26745255-26745328:-    | 1                 | 65                | 384       | 57        |
| tRNA-Ile-AAT-9-1     | Ile-AAT   | chr6:27241739-27241812:+    | 1                 | 31                | 336       | 12        |
| tRNA-Leu-AAG-1-1     | Leu-AAG   | chr5:180524474-180524555:-  | 1                 | 196               | 113       | 33        |
| tRNA-Leu-AAG-2-1     | Leu-AAG   | chr5:180614701-180614782:+  | 1                 | 187               | 137       | 36        |

|                       |         |                             |     |      |      |     |
|-----------------------|---------|-----------------------------|-----|------|------|-----|
| tRNA-Leu-AAG-3-1      | Leu-AAG | chr6:28956779-28956860:+    | 1   | 237  | 122  | 36  |
| tRNA-Leu-AAG-4-1      | Leu-AAG | chr6:28446400-28446481:-    | 1   | 156  | 108  | 25  |
| tRNA-Leu-CAA-1-2      | Leu-CAA | chr6:28908830-28908934:+    | 1   | 129  | 43   | 9   |
| tRNA-Leu-CAA-2-1      | Leu-CAA | chr6:27573417-27573524:-    | 1   | 154  | 34   | 8   |
| tRNA-Leu-CAA-3-1      | Leu-CAA | chr6:27570348-27570454:-    | 1   | 178  | 36   | 9   |
| tRNA-Leu-CAA-4-1      | Leu-CAA | chr1:249168054-249168159:+  | 1   | 158  | 34   | 8   |
| tRNA-Leu-TAG-1-1      | Leu-TAG | chr17:8023632-8023713:-     | 1   | 197  | 113  | 33  |
| tRNA-Leu-TAG-2-1      | Leu-TAG | chr14:21093529-21093610:+   | 1   | 185  | 135  | 34  |
| tRNA-Lys-CTT-1-1      | Lys-CTT | chr14:58706613-58706685:-   | 2   | 76   | 501  | 34  |
| tRNA-Lys-CTT-2-1      | Lys-CTT | chr1:145395522-145395594:-  | 1   | 92   | 496  | 35  |
| tRNA-Lys-CTT-3-1      | Lys-CTT | chr16:3207406-3207478:-     | 1   | 69   | 484  | 30  |
| tRNA-Lys-CTT-4-1      | Lys-CTT | chr16:3241501-3241573:+     | 3   | 71   | 507  | 37  |
| tRNA-Lys-CTT-5-1      | Lys-CTT | chr16:3230555-3230627:-     | 1   | 57   | 423  | 22  |
| tRNA-Lys-CTT-chr7-30  | Lys-CTT | chr7:96770888-96770952:-    | 1   | 41   | 402  | 20  |
| tRNA-Lys-TTT-1-1      | Lys-TTT | chr16:73512216-73512288:-   | 1   | 123  | 156  | 19  |
| tRNA-Lys-TTT-2-1      | Lys-TTT | chr11:122430655-122430727:+ | 1   | 126  | 163  | 21  |
| tRNA-Lys-TTT-3-1      | Lys-TTT | chr1:204475655-204475727:+  | 1   | 123  | 91   | 19  |
| tRNA-Lys-TTT-5-1      | Lys-TTT | chr11:59323902-59323974:+   | 1   | 107  | 85   | 14  |
| tRNA-Lys-TTT-6-1      | Lys-TTT | chr6:27302769-27302841:-    | 1   | 107  | 59   | 11  |
| tRNA-Met-CAT-1-1      | Met-CAT | chr8:124169470-124169542:-  | 1   | 64   | 498  | 105 |
| tRNA-Met-CAT-6-1      | Met-CAT | chr16:87417628-87417700:-   | 1   | 52   | 231  | 78  |
| tRNA-Pro-AGG-1-1      | Pro-AGG | chr16:3241989-3242060:+     | 50  | 2743 | 303  | 253 |
| tRNA-Pro-TGG-1-1      | Pro-TGG | chr14:21101165-21101236:+   | 59  | 3210 | 334  | 240 |
| tRNA-Pro-TGG-3-1      | Pro-TGG | chr5:180615854-180615925:-  | 59  | 3283 | 343  | 282 |
| tRNA-Ser-AGA-chr11-8  | Ser-AGA | chr11:103274966-103275035:+ | 46  | 100  | 1170 | 217 |
| tRNA-Tyr-GTA-chr1-127 | Tyr-GTA | chr1:566376-566441:-        | 288 | 182  | 426  | 569 |
| tRNA-Tyr-GTA-chr14-8  | Tyr-GTA | chr14:32954018-32954083:+   | 280 | 163  | 398  | 527 |
| tRNA-Tyr-GTA-chr21-2  | Tyr-GTA | chr21:10492972-10493037:-   | 288 | 182  | 426  | 569 |
| tRNA-Val-CAC-10-1     | Val-CAC | chr1:17006501-17006573:-    | 1   | 149  | 71   | 14  |

## DISCUSSION

Enhanced protein synthesis and proliferation are properties of tumor cells. Even though the translation machinery components were found to be dysregulated in cancer, the role of tRNAs has not been established well. Recently, genome-wide expressions of differential tRNAs in breast cancer were investigated using high throughput technologies. Functional and phenotypic characterization has been performed by transcriptome analyses. Therefore, tRNAs are believed to play important roles in the regulation and progression of breast cancer and they can be expected as promising prognostic biomarkers (11).

In our study we investigated the *MYC*-dependent deregulated expressions of tRNAs in breast cancer cells by using next-generation sequencing and bioinformatic analysis revealed that 6 out of 71 tRNAs were nuclear-encoded mt-tRNAs.

In the tRNA nomenclature, tRNA-Amino acid refers to the type of tRNA to be charged with an amino acid (28,29). Our results

have shown that nmt-tRNA-Gln anticodon CTG and TTG were found to be deregulated. Sangha et al. demonstrated that tRNA<sup>Gln</sup> showed a median decrease and was downregulated in several cancers (30). We observed that tRNA<sup>Ser</sup> was deregulated depending on *MYC*. Serine tRNAs (tRNA<sup>Ser</sup>) are found to be overexpressed in breast tumors resulting in poor prognosis and an increased risk of recurrence (31).

Our results also demonstrated that upon *MYC* overexpression the level of tRNA<sup>Leu</sup> was downregulated and vice versa for *MYC* inhibition. Leucyl-tRNA synthetase was reported as a tumor suppressor in breast cancer (32). Despite the fact that *MYC* amplification is correlated with malignant phenotypes it masks the effect of the tumor suppressor as for tRNA<sup>Leu</sup>.

Although RNAseq approaches have many advantages like the characterization of transcriptomes, there are also some limitations especially in providing an absolute quantification for the transcripts. With the advance of small RNA sequencing,



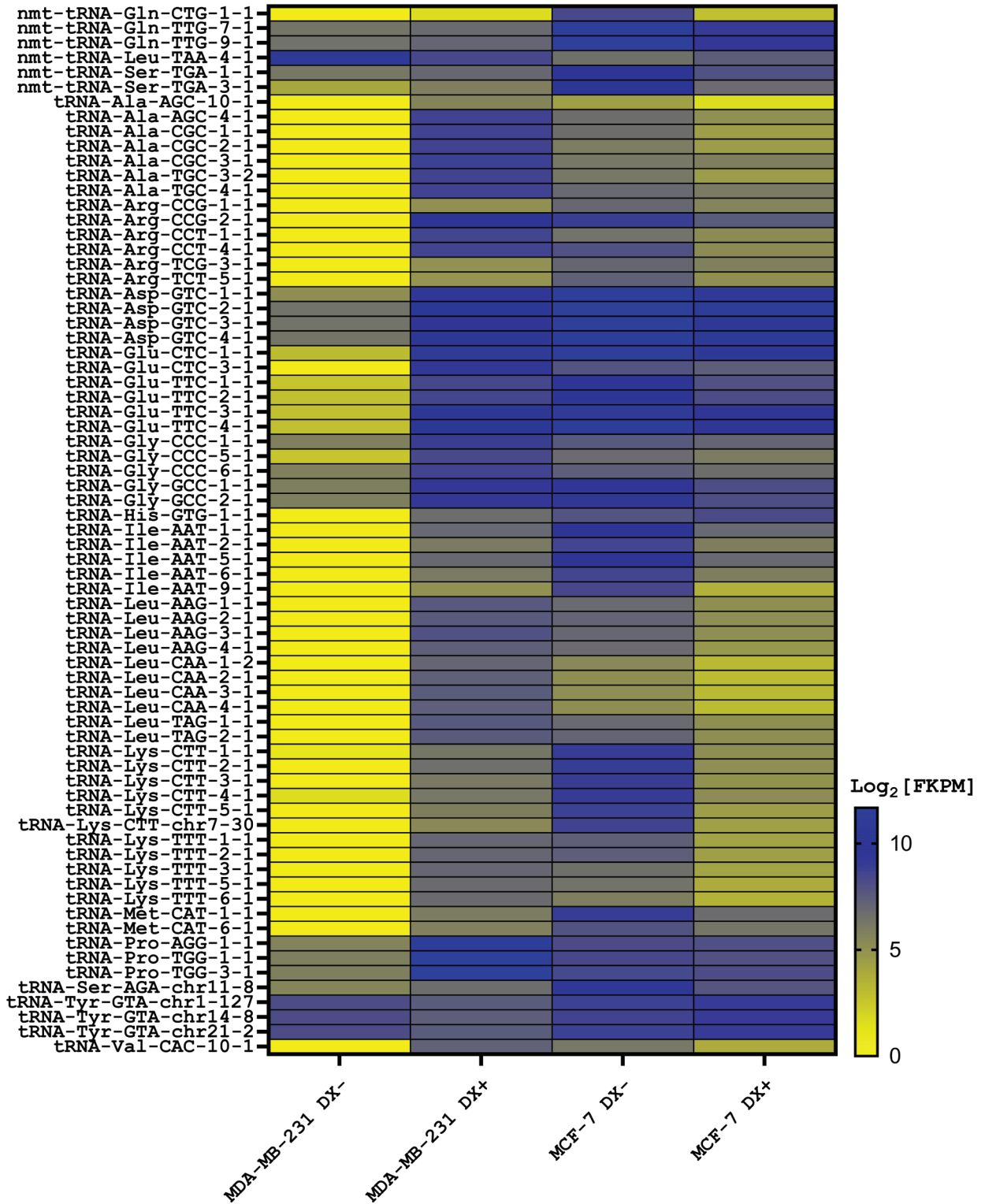


Figure 2: HeatMap Analysis of detected tRNAs in breast cancer cells

it is possible to quantify tRNA gene expression but the limitation is that because of the complexity of tRNA structure sequencing, biases can arise, and also it could be challenging to discriminate reads from mature tRNAs or precursor tRNAs. But nevertheless, small RNA sequencing data are in use for tRNA expressions (33,34).

This study reveals the deregulated expressions of tRNAs including nuclear-encoded mt tRNAs in breast cancer depending on *MYC* overexpression/inhibition. Functional studies are required for underlying molecular mechanisms.

---

**Author Contributions:** Conception/Design of Study- P.E.T., O.T.; Data Acquisition- P.E.T.; Data Analysis/Interpretation- P.E.T., O.T.; Drafting Manuscript- P.E.T., O.T.; Critical Revision of Manuscript- P.E.T., O.T.; Final Approval and Accountability- P.E.T., O.T.; Material and Technical Support- P.E.T., O.T.; Supervision- P.E.T., O.T.

**Peer Review:** Externally peer-reviewed.

**Conflict of Interest:** Authors declared no conflict of interest.

**Financial Disclosure:** This study is supported by Pamukkale University Scientific Research Department (Project no: 2016HZDP006).

## REFERENCES

- Gupta T, Malkin MG, Huang S. tRNA function and dysregulation in cancer. *Front Cell Dev Biol* 2022;1;10:886642.
- Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin J, et al. Sequence and organization of the human mitochondrial genome. *Nature* 1981;290(5806):457-65.
- Montoya J, Christianson T, Levens D, Rabinowitz M, Attardi G. Identification of initiation sites for heavy-strand and light-strand transcription in human mitochondrial DNA. *Proc Natl Acad Sci USA* 1982;79(23):7195-9;
- Ojala D, Montoya J, Attardi G. tRNA punctuation model of RNA processing in human mitochondria. *Nature* 1981;290(5806):470-4.
- Takaku H, Minagawa A, Takagi M, Nashimoto M. A candidate prostate cancer susceptibility gene encodes tRNA 3' processing endoribonuclease. *Nucleic Acids Res* 2003;31(9):2272-8
- Gagliardi D, Stepien PP, Temperley RJ, Lightowlers RN, Chrzanoska-Lightowlers ZM. Messenger RNA stability in mitochondria: different means to an end. *Trends Genet* 2004;20(6):260-7.
- Holzmann J, Frank P, Löffler E, Bennett KL, Gerner C, Rossmanith W. RNase P without RNA: identification and functional reconstitution of the human mitochondrial tRNA processing enzyme. *Cell* 2008;135(3):462-74
- Koripella RK, Sharma M, Risteff P, Keshavan P, Agrawal RK. Structural insights into unique features of the human mitochondrial ribosome recycling. *Proc Natl Acad Sci U.S.A.* 2019;116(17):8283-8
- De Silva D, Tu YT, Amunts A, Fontanesi F, Barrientos A. Mitochondrial ribosome assembly in health and disease. *Cell Cycle* 2015;14(14):2226-50
- Kwon NH, Lee JY, Kim S. Role of tRNAs in Breast Cancer Regulation. *Adv Exp Med Biol* 2021;1187:121-45
- Pavon-Eternod M, Gomes S, Geslain R, Dai Q, Rosner MR, Pan T. tRNA over-expression in breast cancer and functional consequences. *Nucleic Acids Res* 2009;37(21):7268-80.
- Ferrari A, Del'olio S, Barrientos A. The diseased mitoribosome. *FEBS Lett* 2020;595(8):1025-61.
- Kummer E, Ban N. Mechanisms and regulation of protein synthesis in mitochondria. *Nat Rev Mol Cell Biol* 2021;22(5):307-25.
- Cheung EC, Vousden KH. The role of ROS in tumour development and progression. *Nat Rev Cancer* 2022;22(5):280-97.
- Park SG, Schimmel P, Kim S. Aminoacyl tRNA synthetases and their connections to disease. *Proc Natl Acad Sci U S A* 2008;105(32):11043-9.
- Hyeon DY, Kim JH, Ahn TJ, Cho Y, Hwang D, Kim S. Evolution of the multi-tRNA synthetase complex and its role in cancer. *J Biol Chem* 2019;294(14):5340-51.
- Green AR, Aleskandarany MA, Agarwal D, Elsheikh S, Nolan CC, Diez-Rodriguez M, et al. *MYC* functions are specific in biological subtypes of breast cancer and confers resistance to endocrine therapy in luminal tumours. *Br J Cancer* 2016;114(8):917-28.
- Tokgun O, Tokgun PE, Inci K, Akca H. lncRNAs as potential targets in small cell lung cancer: *MYC* -dependent regulation. *Anticancer Agents Med Chem* 2020;20(17):2074-81.
- Dardenne E, Beltran H, Benelli M, Gayvert K, Berger A, Puca L, et al. N-Myc Induces an EZH2-Mediated Transcriptional Program Driving Neuroendocrine Prostate Cancer. *Cancer Cell* 2016;30(4):563-77.
- Xu J, Chen Y, Olopade OI. *MYC* and Breast Cancer. *Genes Cancer* 2010;1(6):629-40.
- Dang CV. *MYC*, metabolism, cell growth, and tumorigenesis. *Cold Spring Harb Perspect Med* 2013;3(8):a014217.
- Kim J, Lee JH, Iyer VR. Global identification of *Myc* target genes reveals its direct role in mitochondrial biogenesis and its E-box usage in vivo. *PLoS One* 2008;3(3):e1798.
- Seitz V, Butzhammer P, Hirsch B, Hecht J, Gütgemann I, Ehlers A, Lenze D, Oker E, Sommerfeld A, von der Wall E, König C, Zinser C, Spang R, Hummel M. Deep sequencing of *MYC* DNA-binding sites in Burkitt lymphoma. *PLoS One* 2011;6(11):e26837.
- Dang CV. *MYC*, microRNAs and glutamine addiction in cancers. *Cell Cycle* 2009;8(20):3243-5.
- Zirin J, Ni X, Sack LM, Yang-Zhou D, Hu Y, Brathwaite R, Bulyk ML, Elledge SJ, Perrimon N. Interspecies analysis of *MYC* targets identifies tRNA synthetases as mediators of growth and survival in *MYC*-overexpressing cells. *PNAS* 2019;116(29):14614-9.
- Tokgun O, Fiorentino FP, Tokgun PE, Yokota J, Akca H. Design of a Lentiviral Vector for the Inducible Expression of *MYC*: A New Strategy for Construction Approach. *Mol Biotechnol* 2017;59(6):200-6.
- Conesa A, Madrigal P, Tarazona S, Gomez-Cabrero D, Cervera A, McPherson A, Szczesniak MW, Gaffney DJ, Elo LL, Zhang X, Mortazavi A. A survey of best practices for RNA-seq data analysis. *Genome Biol* 2016;17(13):2-19.
- Goodenbour JM, Pan T. Diversity of tRNA genes in eukaryotes. *Nucleic Acids Res* 2006;34(21):6137-46.
- Goodarzi H, Nguyen HC, Zhang S, Dill BD, Molina H, Tavazoie SF. Modulated expression of specific tRNAs drives gene expression and cancer progression. *Cell* 2016;165(6):1416-27
- Sangha AK, Kantidakis T. The aminoacyl-tRNA synthetase and tRNA expression levels are deregulated in cancer and correlate independently with patient survival. *Curr Issues Mol Biol* 2022;44(7):3001-17.

31. Santos M, Fidalgo A, Varanda AS, Soares AR, Almeida GM, Martins D, et al. upregulation of tRNA-Ser-AGA-2-1 promotes malignant behavior in normal bronchial cells. *Front Mol Biosci* 2022;9:809985.
32. Passarelli MC, Pinzaru AM, Asgharian H, Liberti MV, Heissel S, Molina H, Goodarzi H, Tavazoie SF. Leucyl-tRNA synthetase is a tumour suppressor in breast cancer and regulates codon-dependent translation dynamics. *Nat Cell Biol* 2022;24(3):307-15.
33. Hoffmann A, Fallmann J, Vilardo E, Mörl M, Stadler PF, Amman F. Accurate mapping of tRNA reads. *Bioinformatics* 2018;34(7):1116-24.
34. Torres AG, Reina O, Stephan-Otto Attolini C, Ribas de Pouplana L. Differential expression of human tRNA genes drives the abundance of tRNA-derived fragments. *Proc Natl Acad Sci U S A* 2019;116(17):8451-6.