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Original Article

Down-regulation of *RB1* and miR-132 in ductal carcinoma of the breast

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Abstract: Introduction: miR-132-3p acts in normal breast development and its downregulation has been documented in breast cancer. One of the targets of miR-132-3p is *RB1* which is also inactivated in breast cancer. The interactions between *RB1* and miR-132 have been reported in several pathological conditions. We aimed to investigate the correlation between expression levels of miR-132 and *RB1* in ductal carcinoma of the breast. Methods: The study was carried out on tissues obtained from female patients with primary breast cancer. Tumor samples were classified using clinical and pathological data. Following RNA extraction and cDNA synthesis, relative gene expressions in tumors were compared to non-cancerous adjacent tissues. The link between *RB1* and miR-132 was assessed by the correlation coefficient test. Results: Our findings revealed a significant decrease in miR-132 and *RB1* expressions with a ratio of 0.165 and 0.365, respectively. Tumor grade showed an association with miRNA-132 levels. The expression of miR-132 in grade I tumors was almost equal to that of normal adjacent tissues, but was intensely decreased in grades II and III. The correlation analysis showed a small linear association between *RB1* and miR-132 levels. Conclusion: The reduction of miR-132 and *RB1* expression confirmed the tumor-suppressive role of both genes in breast cancer. Considering that *RB1* is one of the miR-132 targets, further studies are required to discover any miRNA-mediated upregulation role for miR-132. Our finding discovered a small linear association between miR-132 and *RB1*, which can be concluded towards their independent function in breast cancer pathogenesis.

Keywords: miRNA-132, RB1, breast cancer, gene expression

Introduction

Breast cancer is the most common and leading cause of cancer-related mortality in women worldwide [1]. Genetic factors involved in the cell cycle and mitosis are widely studied in breast cancer pathogenesis [2]. It has been demonstrated that alterations in the retinoblastoma (RB) tumor suppressor pathway could be a potential risk factor for breast cancer [3]. Inactivation of the *RB1* gene was reported in approximately 20-35% of breast tumors associated with poor disease outcomes [4]. *RB1* gene is located on 13q14.2 and can be inactivated by gene deletions or mutation [5]. Hyperphosphorylation of RB1 protein is one of the key events in the G1/S transition of the cell cycle. It mainly acts as a suppressor of transcriptional components and inhibits the expression of essential genes required for cell division [6]. This is while RB1 itself is regulated by other factors, such as microRNAs (miRNAs). miRNAs are conserved twenty-two base pairs (bps) molecules that are involved in several developmental and cellular processes such as cell cycle, cell proliferation, and tumorigenesis [7]. It has been predicted that they target more than half of the human genome including oncogenes and tumor suppressors [8]. miRNAs are deregulated in many solid tumors, mostly creating a specific signature [9].

miR-132 (MIRN132, hsa-MiR-132) has been originally linked to inflammation and neuronal function. Its corresponding gene is located on 17p13.3 and acts in normal breast development by enhancing epithelial-stromal interactions [10]. Pre-miRNA of miR-132 includes 66 bps in length which generates two forms of mature miRNA-132 named miR-132-5p and miR-132-3p. The latter is mainly considered as miR-132 [11]. The miRDB database

Table 1. The sequence of primers for Quantitative Real-time PCR analysis

Primer name	Sequence
miR132-3p Stem-loop	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGACCA
miR132-3p Forward	GCGTCCCTAACAGTCTACA
miR-132-3p Reverse	GTGCAGGGTCCGAGGTAT
miR16 Stem-loop	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCGCCAA
miR16 Forward	CCGGAGTAGCACGTAAAT
miR16 Reverse	TCGTATCCAGTGCAGGG
RB1 Forward	AGAAGGTCTGCCAACACCAA
RB1 Reverse	TTCTTTTGAGCACACGGTCG
GAPDH Forward	ACACCCACTCCTCCACCTTTG
GAPDH Reverse	TCCACCACCCTGTTGCTGTAG

(http://www.mirdb.org/) lists 673 predicted mRNA targets for miR-132-3p [12]. Among them, *RB1* is one of the few validated targets of miR-132-3p confirmed via low-throughput and high-throughput experiment [13, 14].

Since both molecules play prominent roles in the pathogenesis of breast cancer, we aimed to determine their expression levels in ductal carcinoma of the breast.

Material and methods

Patient specimens

Thirty tissue samples of primary breast tumors along with non-cancerous adjacent tissues (NATs) were collected at the Sina hospital and Farmanieh hospital in Tehran, from April 2018 to August 2019. Inclusion criteria comprise being female, new case of biopsy-confirmed primary breast invasive ductal carcinoma, aware and consent to participate in the study. Exclusion criteria were receiving any chemotherapy, radiotherapy, hormone therapy, previous breast surgery and the presence of any other diseases that can affect the study.

Samples were collected by an expert breast surgeon and divided into two parts: one part was sent directly for pathology examination and the second part was immediately stored in the nitrogen tank until used for RNA extraction.

The tumors were classified by the pathological grade scheme in line with the American joint committee on cancer (AJCC) guideline. Molecular classification of breast tumor subtypes was applied based on estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) statuses, determined by immunohistochemistry (IHC) method. The values of the Ki67 index above 14% were considered positive to discriminate between luminal A and luminal B subtypes, as recommended.

RNA extraction and cDNA synthesis

Total RNA was isolated from the tissue using the Allspin™ mini kit (GeneAll, Pishgam Biotech, Iran) according to manufacturer instructions. The quality and quantity of the total RNAs were measured by NanoPhotometer (NP80, Germany) and the RNA integrity was confirmed by gel electrophoresis. cDNA synthesis was performed using a 2X RT pre-mix kit (BioFact, NoavaranTeb, Iran). OligodT and random hexamer were simultaneously included to increase cDNA synthesis efficacy.

To achieve a higher specificity in miRNA detection, we used the stem-loop method. The stem-loop was incubated at 95°C for 8 minutes and then at 25°C for 10 minutes. The reaction mixture contained cDNA synthesis pre-mix, stem-loop primer, forward primer, and required values of total RNAs (**Table 1**). The cDNA synthesis reaction was incubated for 30 minutes at 50°C and 5 minutes at 95°C and was kept at -20°C.

Quantitative real-time reverse transcription-PCR (qRT-PCR) assay

Real-time PCR assays were performed using cDNA product, 2X master mix (BioFact, NoavaranTeb, Iran), and specific primers. The GAPDH gene was recruited as an internal control for the RB1 gene and the miR-16 as an internal control of miR-132 (**Table 1**). The reaction was carried out on Step One apparatus

(Applied Biosystems) with the following conditions: 95°C for 15 minutes, followed by 40 cycles of 95°C for 20 seconds, 60°C for 40 seconds, and 72°C for 35 seconds. All amplifications were performed in duplicate and no template control (NTC) was included for each reaction. The level of efficiency for each primer pair was assessed by LinReg PCR software (Academic Medical Centre, Amsterdam, the Netherlands). This software analyzes SYBR green qPCR reactions and subtracts the baseline fluorescence in all samples.

Receiver operating characteristic (ROC) curve

We applied a ROC curve analysis to appraise the sensitivity and specificity of RB1 and miR-132 expression in breast tissue. The significance of gene expression variations was evaluated by IBM Statistical Package for the Social Sciences (SPSS). The area under the ROC curve (AUC), sensitivity, and one minus specificity were calculated to compare the predictive values of the gene expressions.

Statistical analyses

Real-time PCR data analyses were carried out with REST 2009 software (Qiagen, Hilden, Germany) and SPSS software. The expression levels were normalized to internal controls and subsequently, relative expression levels in the tumor tissues were compared to the NATs based on the $2^{-\Delta\Delta Ct}$ method. To study the influence of clinical parameters, relative expression levels were analyzed by the Kolmogorov-Smirnov to check the distribution normality. One-way ANOVA was used for normally distributed data. For the data which were not distributed normally, non-parametric Mann Whitney U test was employed. The study of the correlation between *RB1* and miR-132 gene expression was determined by the correlation coefficient (r^2) using the Pearson test. Data were expressed as mean values ± standard error and were considered statistically significant with $P \leq 0.05$ and 95% confidence intervals (CI).

Results

Clinicopathological characteristics

All the patients were new cases of breast ductal carcinoma with a mean age of 59.7 ± 13 ranging from 43 to 82 years old. The mean tumor sizes were 2.55 ± 1.52 cm (range, 0.7-7 cm). Based on the histopathological results, 20.3% of the tumors were grade I, 53% grade II and 26.7% grade III. The IHC results revealed that 20% and 87% of tumors were HER2 positive and ER/PR positive, respectively. Additional Ki67 index assessment regarding molecular subtype's classification showed that luminal A, luminal B, HER2 positive, and basal-like comprised 33.3%, 53.3%, 6.7%, and 6.7% of tumors, respectively.

RB1 and miR-132 were down-regulated in breast tumors

The amplification of the interest genes and internal controls resulted in the corresponding desired fragments. The accuracy of PCR amplification was determined by agarose gel electrophoresis and the presence of single, sharp melting curves (**Figure 1**). The *RB1* and miR-132 PCR efficiency were 1.99 and 1.86 obtained by the LinRegPCR software.

The relative gene expression levels of *RB1* and miR-132 were quantified comparative to their expression in NATs, following normalization to the GAPDH and miR-16 internal controls, respectively. We observed a significant down-regulation of *RB1* and miR-132 levels in tumor samples in comparison to matched NATs. The relative expression levels were calculated at 0.365 (*P*-value = 0.044) for the *RB1* gene. The analysis results regarding miR-132 showed relative expression levels of 0.165 with a *P*-value of 0.028 (**Figure 2**).

Discrimination power of RB1 and miR-132

By drawing the ROC curves, we analyzed the selection power of the genes. As shown in **Figure 3**, the RB1 and miR-132 discriminate breast cancer with AUC of 0.657 and 0.668, respectively. The 95% confidence interval of the AUC was 0.519-0.795 for RB1, with a Std. Error of 0.071. The ROC analysis of miR-132 displayed a confidence interval of 0.524-0.813 and Std. Error of 0.074. The results indicated the same discrimination power for RB1 and miR-132.

Correlated expression of RB1 and miR-132

The matched expression of *RB1* and miR-132 was checked via http://www.mirdb.org/ database by calculating target score. The in-silico data showed that *RB1* is one of the predicted

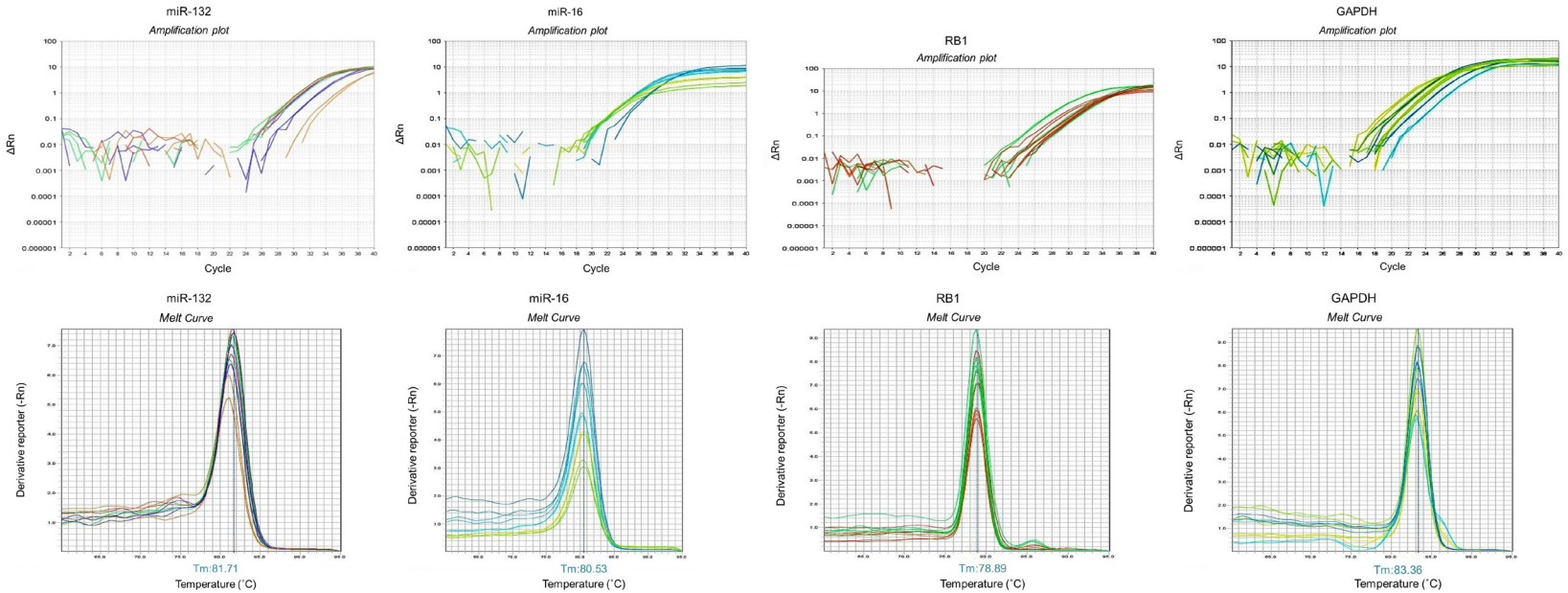


Figure 1. Amplification plot and melt curve of target and control genes.

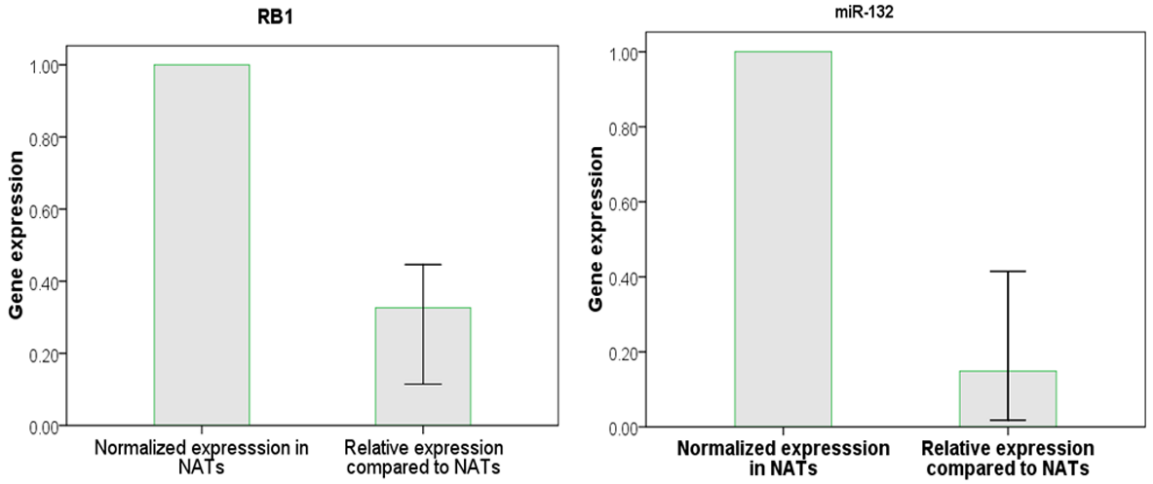


Figure 2. RB1 and miR-132 relative gene expression; the bar plots signify expression in the tumor compared to NATS.

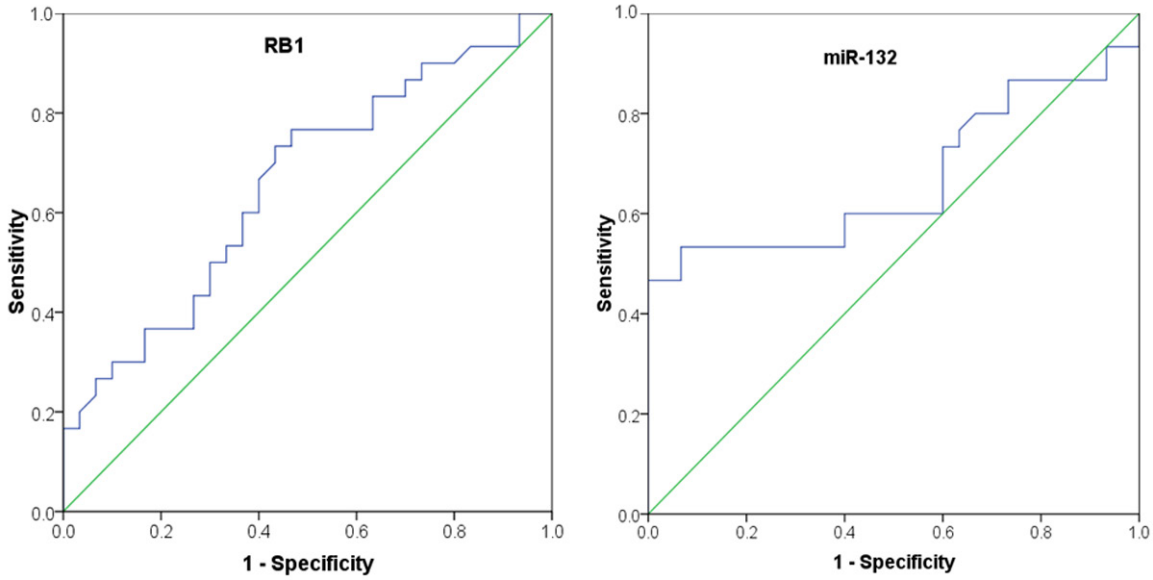
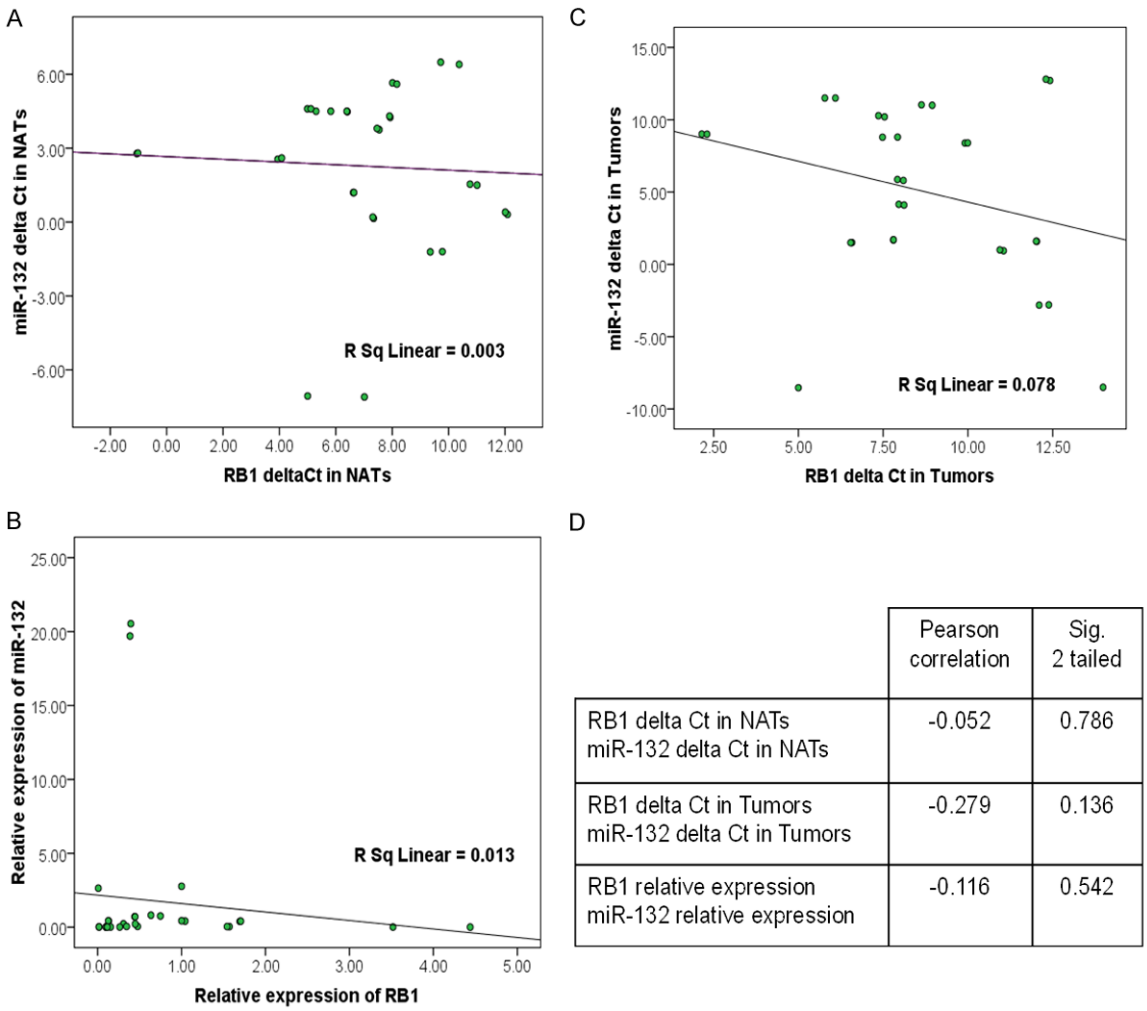


Figure 3. ROC curve analysis to evaluate the performance of *RB1* and miR-132.

targets for hsa-miR-132-3p with a high score of 90. The statistical evaluation of the correlation between *RB1* and miR-132 gene expression was conducted by the Pearson test. **Figure 4** shows Pearson Correlation plots for *RB1*/miR-132 matched expression in tumor tissues as well as in NATs. The relative gene expressions ($2^{-\Delta\Delta Ct}$) plot for *RB1*/miR-132 is also presented in **Figure 4**. The statistical analysis indicated no significant correlation between the two RNA levels. The r^2 was calculated at 0.003 and 0.078 in NATs and tumor tissues, respectively. The r^2 was 0.013 in correlation between the relative gene expressions of the two RNAs.

Correlation between tumor grades and the miR-132 expression

To inspect whether *RB1* and/or miR-132 were correlated to patients' clinicopathological parameters, we analyzed relative gene expression levels against tumor grade, size, subtypes, and clinical stage as well as ER, PR, and HER2 status. The obtained results showed a statistically significant correlation between miR-132 and tumor grade with a *P*-value of 0.04. **Figure 5** shows that in grade I, the ratio of miR-132 to the NATs was around 1.2. However, in grades II and III, the ratios were greatly decreased to



	Pearson correlation	Sig. 2 tailed
RB1 delta Ct in NATs miR-132 delta Ct in NATs	-0.052	0.786
RB1 delta Ct in Tumors miR-132 delta Ct in Tumors	-0.279	0.136
RB1 relative expression miR-132 relative expression	-0.116	0.542

Figure 4. Pearson Correlation plots for miR-132/RB1 matched expression. (A) ΔCt in NATs, (B) ΔCt in tumor tissue, (C) Relative expression ($2^{-\Delta\Delta Ct}$), (D) Statistical results of the correlation analysis.

less than 0.2. Statistical analyzes did not show a significant link between miR-132 and other parameters. Also, no significant correlation was observed between *RB1* expression and clinicopathological features including tumor grades (**Figure 5**).

Discussion

The presence of the miR-132 gene on 17p13.3, a genomic location that is frequently lost in malignancies, underscores its importance in cancer studies [15]. Although a few reports indicated increase in miR-132 expression in cancers [16-18], most studies including prostate cancer [19], colorectal cancer [20, 21] oral squamous cell carcinoma [22] and lung cancer [23, 24] described its down-regulation.

In breast cancer, further support of a tumor suppressor role has been suggested based on evidence of decreased miR-132 levels [25-27]. A study on patients' survival displayed that the expression of miR-132 was even lower in brain metastasis than in their paired breast tumors [28].

Several mechanisms have been reported for the mode of action of miR-132 in breast tumors. One of them is to target forkhead box protein A1 (FOXA1), which acts in favor of breast cancer progression [25]. In another study, hematological and neurological expressed 1 (HN1) mRNA was described as a direct target of miR-132. By inactivating HN1, which promotes the proliferation and invasion of tumor cells, miR-132 could inhibit breast cancer [27].

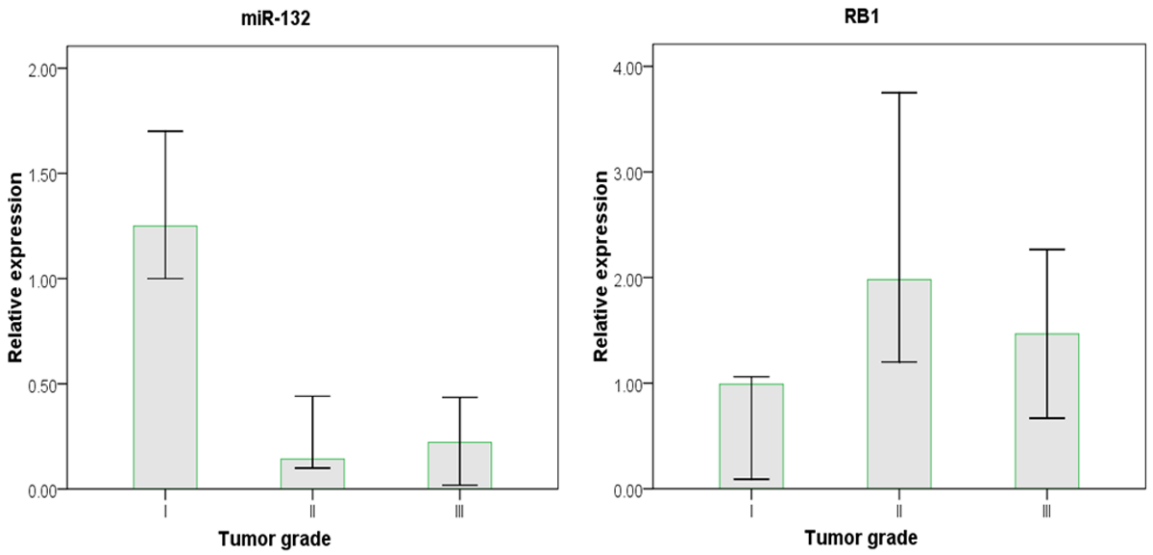


Figure 5. The relative expression levels of miR-132 indicates significant decrease in grades II and III. The relative expression levels of *RB1* in different tumor grades did not show an interpretable pattern.

Using luciferase reporter assay, western blot, and real-time PCR, it was confirmed that miR-132 can directly target the mRNA of glycogen synthase kinase-3β (GSK-3β) and reduce its protein expression. GSK3B plays multiple roles in cell signaling and proliferation [29].

We noticed that the expression of miR-132 was intensely decreased in breast tumors compared to NATs of the same patients. In accordance with another study conducted on the Iranian population [26], we detected a more robust down-regulation in the tumors exhibiting higher pathological grades. However, no significant difference was observed in the grade I tumor. Björner and colleagues performed an investigation on early precancerous breast lesions known as the columnar cell hyperplasia (CCH) in comparison to invasive breast cancer. They described a three-fold increase in miR-132 in the stroma surrounding CCH but the lower levels of the stromal miR-132 in invasive breast cancer. This reduction was not only lower than CCH but also than stroma surrounding normal cells, indicating that miR-132 was over-expressed in the primary lesions but was reduced by the tumor progression. They also explored differentially expressed target genes by *in-vitro* overexpression of miR-132 in human mammary fibroblasts. However, *RB1* was not among 10 mostly up or down-regulated genes following miR-132 induction [30]. This evidence in addition to our finding that indicates a small correlation between RB1 and miR-132, raise the prospect of their independent function in breast tumors.

The cellular interaction between *RB1* and miR-132 was first reported in gastric cancer by Gao and colleagues. Using luciferase reporter assays they confirmed that by up-regulation of miR-132, *RB1* expression was inhibited. Since *RB1* over-expression blocked the expression and proliferative effects of miR-132, a reciprocal effect between RB1 and miR-132 was suggested [18]. The inverse link between RB1 and miR-132 has also been reported by Park and colleagues. By *in-vitro* miR-132 suppression, they described an *RB1* increased expression in pancreatic cancer [14]. However, data analysis of the pancreatic ductal adenocarcinoma from the cancer genome atlas (TCGA) showed convergent expression of *RB1* and miR-132 [31].

Bijkerk and colleagues revealed more complexity of miR-132 and RB1 correlation depending on the cell type and condition. In the mice model of kidney fibrosis treated with antagomir-132, they identified increased phosphorylated RB1 protein in kidney lysates as an indicator of increased cell proliferation. However, by analyzing the expression of RB1 gene in the interstitial cells from antagomir-132 treated mice compared with scramblemir-treated mice, they detected no change in RB1 mRNA [32].

How miR-132 and RB1 relate to the progression of cancer is a question that needs more research, to be answered. Most studies, especially those in breast cancer, have reported decreased expression for both genes, indicating that *RB1* is not inhibited by miR-132. However, as mentioned earlier, bioinformatic analyzes strongly identify RB1 as one of the main targets of miR-132. A possible speculation could be enhancing effects of miR-132 on *RB1* such as miRNA-mediated upregulation. In that case, as the tumor progresses and miR-132 decreases so does *RB1*. This suggestion was reinforced by the study that found a cell cycle inhibitory role for miR-132 in osteosarcoma cells. In vitro overexpression of miR-132 caused G1/S arrest that subsequently suppressed cell proliferation and tumor growth [33].

The application of cell cycle inhibitors in breast cancer has brought more attention to RB1 and miR-132. The role of *RB1* has been identified in response to CDK4/6 inhibitors which currently are used in the treatment of metastatic hormone receptor-positive, HER2-negative breast cancers [34]. New results indicate that in the presence of *RB1* expression, CDK4/6 inhibitors enhance the radio-sensitivity of tumors including triple-negative breast cancers [35].

Conclusions

Taken together, we observed a substantial decrease in expression levels of miRNA-132 and *RB1* in breast ductal carcinoma compared to NATs tissues. The miR-132 did not show a significant decrease in grade I tumors, but with the progression of the tumor to grade II and III, a more pronounced decrease was observed. Our study revealed a small linear association between *RB1* and miR-132 that emphasized the possibility of an independent function of miR-132 and *RB1* in breast cancer. Given the importance of both genes in breast cancer pathogenesis, further studies on their cross-talk in tumor initiation and progression could lead toward developing therapies.

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Informed consent was obtained from all individual participants included in the study.

Disclosure of conflict of interest

None.

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References

[1] Ferlay J, Colombet M, Soerjomataram I, Parkin DM, Piñeros M, Znaor A and Bray F. Cancer statistics for the year 2020: an overview. Int J Cancer 2021; 149: 778-789.

[2] Mohamadalizadeh-Hanjani Z, Shahbazi S and Geranpayeh L. Investigation of the SPAG5 gene expression and amplification related to the NuMA mRNA levels in breast ductal carcinoma. World J Surg Oncol 2020; 18: 225.

[3] Derenzini M, Montanaro L, Vici M, Barbieri S, Ceccarelli C, Santini D, Taffurelli M, Martinelli GN and Trere D. Relationship between the RB1 mRNA level and the expression of phosphorylated RB protein in human breast cancers: their relevance in cell proliferation activity and patient clinical outcome. Histol Histopathol 2007; 22: 505-513.

[4] Risi E, Grilli A, Migliaccio I, Biagioni C, McCartney A, Guarducci C, Bonechi M, Benelli M, Vitale S, Biganzoli L, Bicciato S, Di Leo A and Malorni L. A gene expression signature of retinoblastoma loss-of-function predicts resistance to neoadjuvant chemotherapy in ER-positive/HER2-positive breast cancer patients. Breast Cancer Res Treat 2018; 170: 329-341.

[5] Yao Y, Gu X, Xu X, Ge S and Jia R. Novel insights into RB1 mutation. Cancer Lett 2022; 547: 215870.

[6] Blais A and Dynlacht BD. E2F-associated chromatin modifiers and cell cycle control. Curr Opin Cell Biol 2007; 19: 658-662.

[7] Bartel DP. MicroRNAs: target recognition and regulatory functions. Cell 2009; 136: 215-233.

[8] Kozomara A, Birgaoanu M and Griffiths-Jones S. miRBase: from microRNA sequences to function. Nucleic Acids Res 2019; 47: D155-D162.

[9] Volinia S, Calin GA, Liu CG, Ambs S, Cimmino A, Petrocca F, Visone R, Iorio M, Roldo C, Ferracin M, Prueitt RL, Yanaihara N, Lanza G, Scarpa A, Vecchione A, Negrini M, Harris CC and Croce CM. A microRNA expression signature of human solid tumors defines cancer gene targets. Proc Natl Acad Sci U S A 2006; 103: 2257-2261.

[10] Ucar A, Vafaizadeh V, Jarry H, Fiedler J, Klemmt PA, Thum T, Groner B and Chowdhury K. miR-212 and miR-132 are required for epithelial stromal interactions necessary for mouse mammary gland development. Nat Genet 2010; 42: 1101-1108.
[11] Qian Y, Song J, Ouyang Y, Han Q, Chen W, Zhao X, Xie Y, Chen Y, Yuan W and Fan C. Advances in roles of miR-132 in the nervous system. Front Pharmacol 2017; 8: 770.
[12] Chen Y and Wang X. miRDB: an online database for prediction of functional microRNA targets. Nucleic Acids Res 2020; 48: D127-D131.
[13] Haviv R, Oz E and Soreq H. The stress-responding miR-132-3p shows evolutionarily conserved pathway interactions. Cell Mol Neurobiol 2018; 38: 141-153.
[14] Park JK, Henry JC, Jiang J, Esau C, Gusev Y, Lerner MR, Postier RG, Brackett DJ and Schmittgen TD. miR-132 and miR-212 are increased in pancreatic cancer and target the retinoblastoma tumor suppressor. Biochem Biophys Res Commun 2011; 406: 518-523.
[15] Stack M, Jones D, White G, Liscia DS, Venesio T, Casey G, Crichton D, Varley J, Mitchell E, Heighway J, et al. Detailed mapping and loss of heterozygosity analysis suggests a suppressor locus involved in sporadic breast cancer within a distal region of chromosome band 17p13.3. Hum Mol Genet 1995; 4: 2047-2055.
[16] Wu HY, Xia S, Liu AG, Wei MD, Chen ZB, Li YX, He Y, Liao MJ, Hu QP and Pan SL. Upregulation of miR-132-3p in cholangiocarcinoma tissues: a study based on RT-qPCR, The Cancer Genome Atlas miRNA sequencing, gene expression omnibus microarray data and bioinformatics analyses. Mol Med Rep 2019; 20: 5002-5020.
[17] Bu X, Zhang J, Tian F, Wang X, Wu L and Tian W. Value of diffusion-weighted magnetic resonance imaging combined with miR-18a level in predicting radiosensitivity of cervical cancer. Med Sci Monit 2018; 24: 7271-7278.
[18] Gao FY, Liu QY, Yuan L and Xuan SY. Upregulation of microRNA-132 in gastric cancer promotes cell proliferation via retinoblastoma 1 targeting. Mol Med Rep 2015; 12: 7005-7010.
[19] Qu W, Ding SM, Cao G, Wang SJ, Zheng XH and Li GH. miR-132 mediates a metabolic shift in prostate cancer cells by targeting Glut1. FEBS Open Bio 2016; 6: 735-741.
[20] Zheng YB, Luo HP, Shi Q, Hao ZN, Ding Y, Wang QS, Li SB, Xiao GC and Tong SL. miR-132 inhibits colorectal cancer invasion and metastasis via directly targeting ZEB2. World J Gastroenterol 2014; 20: 6515-6522.
[21] He X, Ma J, Zhang M, Cui J and Yang H. Long non-coding RNA SNHG16 activates USP22 expression to promote colorectal cancer progression by sponging miR-132-3p. Onco Targets Ther 2020; 13: 4283-4294.
[22] Chen L, Zhu Q, Lu L and Liu Y. MiR-132 inhibits migration and invasion and increases chemosensitivity of cisplatin-resistant oral squamous cell carcinoma cells via targeting TGF-β1. Bioengineered 2020; 11: 91-102.
[23] Li Y, Zu L, Wang Y, Wang M, Chen P and Zhou Q. miR-132 inhibits lung cancer cell migration and invasion by targeting SOX4. J Thorac Dis 2015; 7: 1563-1569.
[24] Su Y, Shetty A and Jiang F. Integrated analysis of miRNAs and DNA methylation identifies miR-132-3p as a tumor suppressor in lung adenocarcinoma. Thorac Cancer 2020; 11: 2112-2124.
[25] Wang D, Ren J, Ren H, Fu JL and Yu D. MicroRNA-132 suppresses cell proliferation in human breast cancer by directly targeting FOXA1. Acta Pharmacol Sin 2018; 39: 124-131.
[26] Damavandi Z, Torkashvand S, Vasei M, Soltani BM, Tavallaei M and Mowla SJ. Aberrant expression of breast development-related microRNAs, miR-22, miR-132, and miR-212, in breast tumor tissues. J Breast Cancer 2016; 19: 148-155.
[27] Zhang ZG, Chen WX, Wu YH, Liang HF and Zhang BX. MiR-132 prohibits proliferation, invasion, migration, and metastasis in breast cancer by targeting HN1. Biochem Biophys Res Commun 2014; 454: 109-114.
[28] Giannoudis A, Clarke K, Zakaria R, Varešlija D, Farahani M, Rainbow L, Platt-Higgins A, Ruthven S, Brougham KA, Rudland PS, Jenkinson MD, Young LS, Falciani F and Palmieri C. A novel panel of differentially-expressed microRNAs in breast cancer brain metastasis may predict patient survival. Sci Rep 2019; 9: 18518.
[29] Xu C, Du Z, Ren S and Pian Y. Downregulation of GSK3B by miR-132-3p enhances etoposide-induced breast cancer cell apoptosis. Ann Clin Lab Sci 2021; 51: 285-294.
[30] Björner S, Fitzpatrick PA, Li Y, Allred C, Howell A, Ringberg A, Olsson H, Miller CJ, Axelson H and Landberg G. Epithelial and stromal microRNA signatures of columnar cell hyperplasia linking Let-7c to precancerous and cancerous breast cancer cell proliferation. PLoS One 2014; 9: e105099.
[31] Chen Y, Zhu H, Wang Y, Song Y, Zhang P, Wang Z, Gao J, Li Z and Du Y. MicroRNA-132 plays an independent prognostic role in pancreatic ductal adenocarcinoma and acts as a tumor suppressor. Technol Cancer Res Treat 2019; 18: 1533033818824314.
[32] Bijkerk R, de Bruin RG, van Solingen C, van Gils JM, Duijs JM, van der Veer EP, Rabelink TJ, Humphreys BD and van Zonneveld AJ. Silenc-

ing of microRNA-132 reduces renal fibrosis by selectively inhibiting myofibroblast proliferation. Kidney Int 2016; 89: 1268-1280.

[33] Wang J, Xu G, Shen F and Kang Y. miR-132 targeting cyclin E1 suppresses cell proliferation in osteosarcoma cells. Tumour Biol 2014; 35: 4859-4865.

[34] Roberto M, Astone A, Botticelli A, Carbognin L, Cassano A, D'Auria G, Fabbri A, Fabi A, Gamucci T, Krasniqi E, Minelli M, Orlandi A, Pantano F, Paris I, Pizzuti L, Portarena I, Salesi N, Scagnoli S, Scavina P, Tonini G, Vici P and Marchetti P. CDK4/6 inhibitor treatments in patients with hormone receptor positive, Her2 negative advanced breast cancer: potential molecular mechanisms, clinical implications and future perspectives. Cancers (Basel) 2021; 13: 332.

[35] Pesch AM, Hirsh NH, Michmerhuizen AR, Jungles KM, Wilder-Romans K, Chandler BC, Liu M, Lerner LM, Nino CA, Ward C, Cobain EF, Lawrence TS, Pierce LJ, Rae JM and Speers CW. RB expression confers sensitivity to CDK4/6 inhibitor-mediated radiosensitization across breast cancer subtypes. JCI Insight 2021; 7: e154402.