**in vivo** *36*: 1497-1502 (2022)
doi:10.21873/invivo.12857

# Role of MicroRNA-31 (miR-31) in Breast Carcinoma Diagnosis and Prognosis

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**Abstract.** *Background/Aim: Breast cancer (BC) is among the most widespread malignant tumors in women. In the current study, we evaluated the role of miR-31 in BC patients and its relation to the different prognostic, clinical, and pathological features. Patients and Methods: MiR-31 levels were determined by RT-PCR in BC and adjacent normal breast tissues from 100 BC patients. BC diagnosis was established through histopathological examinations. The expression of estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) receptor in all tumors was determined using immunohistochemistry. Results: MiR-31 expression was reduced in BC tissues relative to adjacent healthy breast tissue (mean levels were 0.93 and 7.2, respectively). Also, the low expression of miR-31 in BC patients was significantly correlated with adverse clinical and pathological features such as: young patient's age, premenopausal status, infiltrative lobular carcinoma, ER and PR negative tumors, HER2 positive tumors, and advanced clinical stage. Conclusion: MiR-31 was expressed at low levels in BC tissues and correlated with adverse clinical and pathological features, and poor survival.*

BC is now the most common malignant tumor in women and the second most prevalent cancer worldwide (1). While the advances in early diagnosis and care have reduced BC mortality rate, the detection and management of BC continue to remain a significant community healthcare issue (2). Breast carcinogenesis is a complex mechanism influenced by genetic and epigenetic changes that affect main cellular mechanisms implicated in proliferation and progression (3). A detailed analysis of the genomic processes engaged in BC induction and development is likely to lead to the detection of valuable biomarkers and molecular triggers for BC management. Micro RNAs are short non-coding RNAs that serve significant functions by modulating the activity of genes involved in transcriptional inhibition or enhancement (4). According to the literature, such micro RNAs (about 20-25 nucleotides chain) are engaged in a wide range of cellular processes including cell division, replication, apoptosis, stress tolerance, and cancer cell spread (5, 6). Among miRNAs, miR-31 has been shown to exert a suppressing or promoting function in cancer (7). Its suppressive function has been shown to be markedly diminished in hepatocellular (HCC), renal carcinoma, and CNS malignancies, while expression of its target genes is enhanced resulting in increased cell division (8, 9). Furthermore, miR-31 expression was significantly increased in pancreatic, colorectal, and lung cancer, and resulted in suppression of the genes regulating significant cellular activities (10, 11). A few studies have suggested that miR-31 is a vital controller of BC metastasis (8, 12). Recent studies have also shown that this microRNA is the primary originator or controller of breast carcinogenesis (7, 13). Only limited studies have explored the correlation between miR-31 and BC. The current study aimed to investigate the impact of miR-31in BC patients and its association with different prognostic, clinical, and pathological features.

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*Key Words:* miRNA-31, breast carcinoma, diagnosis, prognosis.



## Patients and Methods

*Patients.* The current study included 100 BC patients treated at the clinical oncology department, Zagazig University, between November 2015 and October 2020. Tissue specimens from the primary breast tumor and the nearby normal tissues (NNT) were

obtained at surgery and stored in liquid nitrogen for future analysis. None of the patients received any treatment before surgery. The included patients were clinically staged based on the American Joint Committee of Cancer (AJCC) (14). The research design was approved by the University of Zagazig's Ethical Board. All patients gave informed consent before inclusion.

*Quantitative PCR*. The mirVana miRNA isolation kit was utilized to isolate RNA from the tissues according to the manufacturers' instructions (Ambion INC, Redondo, CA, USA). The Poly(A) tailing package (Ambion) was used to attach a poly(A) tail to the RNA. cDNA was sequenced by the TaqMan Reverse Transcription Package (Applied Biosystems, Foster, CA, USA). The PCR was implemented employing the TaqMan Micro-RNA kit Assay (Applied Biosystems). The primers were purchased from Ambion. The miR-31 sequence was: 5'-AGGCAAGATGCTGGCATAGCT-3'; and that of U6 was 5'-TGACACGCAAATTCGTGAAG-3'. The PCR and results processing were conducted using iCycler (Bio-Rad, Hercules, CA, USA). All samples were examined three times. The expression of the miRNA was measured relative to the U6 RNA. The expression levels were estimated using the $2^{-\Delta\Delta Ct}$ method (15). The miR-31 expression levels are shown after normalization to the control (U6).

*Determination of estrogen, progesterone, and human epidermal growth factor receptor 2 receptors*. Expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) in BC tissues or NNT were determined by immunohistochemistry (IHC) on formalin-fixed paraffin-embedded tissues. Rabbit monoclonal primary anti-ER (clone SP1), anti-PR (clone 1E2), and anti- HER2/neu (clone 4B5) antibodies (Invitrogen, Waltham, MA, USA) were used for determination of ER, PR, and HER2, respectively. The semi-automated staining system and reagents of Ventana Benchmark were utilized for staining (Ventana Medical Systems, Basel, Switzerland). Staining analysis was performed based on the guidelines of the American Society of Clinical Oncology (ASCO) and the College of American Pathologists (CAP) (16).

*Statistical analysis*. Analysis of the results was performed by SPSS® version 23.0 (SPSS Inc., Chicago, IL, USA). The results are reported as a mean±SD. Our data were assessed using one-way analysis of variance (ANOVA) and the student's *t*-test. Receiver operating characteristic (ROC) curve was plotted to distinguish between patients with and without breast cancer. Survival of our patients was estimated using the Kaplan-Meier method.

## Results

The study included 100 BC patients aged between 25 and 65 years, and the mean age was 48.5 years. Fifty-one were postmenopausal. The majority of patients were stage IIA. Table I summarizes the patient's clinical characteristics. The PCR evaluation of miR-31 levels in 100 BC and NNT specimens revealed a significant down-regulation of miR-31 in the BC tissues compared to those in the NNT. The mean level was 0.93 ($2^{-\Delta\Delta Ct}$) in cancer tissues compared to 7.2 in normal breast tissues, $p<0.0001$ (Figure 1). The receiver operating characteristic (ROC) curve was plotted to evaluate the ability of miR-31 levels to discriminate between BC and the NNT. A specificity of 100% and a sensitivity of 98% at the cutoff level >3.785 (AUC=0.9989, 95%CI=0.9965 to 1.000, $p<0.0001$) was revealed (Figure 2). Furthermore, the expression of miR-31 was correlated with different clinical and pathological features. Low miR-31 expression corelated with unfavorable prognostic parameters. Low expression was significantly related to young age ($p<0.0001$), premenopausal status ($p<0.0001$), infiltrative lobular carcinoma ($p<0.0001$), grade III and IV tumors ($p<0.0001$), large tumors ($p<0.0001$), and axillary lymph node infiltration ($p<0.0001$). Determination of ER and PR expression using IHC revealed that 26% and 31% of tumors, respectively, were negative. Furthermore, ER and PR negative tumors showed significantly lower expression of miR-31 ($p<0.0001$). However, HER2 positive tumors (16%) showed significantly lower expression of miR-31 (Table II). Kaplan-

Table I. *miR-31 expression in breast cancer patients according to their clinical and pathological characteristics*.

| Parameters | miR-31 | | | *p*-Value |
|---|---|---|---|---|
| | Number | Mean | SD | |
| Age | | | | |
| 20-30 | 8 | 0.03 | 0.02 | <0.0001[a] |
| 31-40 | 17 | 0.09 | 0.02 | |
| 41-50 | 23 | 0.43 | 0.17 | |
| 51-60 | 38 | 1.07 | 0.29 | |
| 61-70 | 14 | 2.92 | 0.29 | |
| Menopausal status | | | | |
| Premenopausal | 49 | 0.25 | 0.22 | <0.0001[b] |
| Postmenopausal | 51 | 1.59 | 0.88 | |
| Pathological type | | | | |
| IDC | 86 | 1.08 | 0.92 | <0.0001[b] |
| ILC | 14 | 0.05 | 0.03 | |
| Pathological grade | | | | |
| I-II | 57 | 1.49 | 0.88 | <0.0001[b] |
| III-IV | 43 | 0.20 | 0.18 | |
| Tumor (T) | | | | |
| T1 | 18 | 2.63 | 0.62 | <0.0001a |
| T2 | 57 | 0.78 | 0.35 | |
| T3 | 20 | 0.09 | 0.03 | |
| T4 | 5 | 0.01 | 0.01 | |
| Nodes (N) | | | | |
| N0 | 22 | 2.41 | 0.73 | <0.0001[a] |
| N1 | 48 | 0.78 | 0.27 | |
| N2 | 30 | 0.09 | 0.06 | |
| Stage | | | | |
| IA | 3 | 3.15 | 0.02 | <0.0001[a] |
| IB | 9 | 2.98 | 0.07 | |
| IIA | 35 | 1.19 | 0.39 | |
| IIB | 26 | 0.52 | 0.18 | |
| IIIA | 24 | 0.09 | 0.04 | |
| IIIB | 3 | 0.01 | 0.00 | |

ILC: Infiltrative lobular carcinoma; IDC: intra-ductal carcinoma. [a]One way analysis of variance (ANOVA); [b]Student's *t*-test.

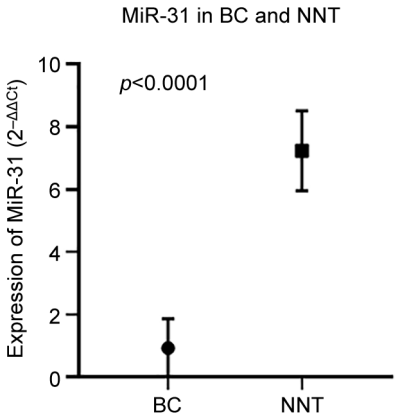


Figure 1. *MiR-31 expression levels in breast cancer (BC) and nearby normal tissues (NNT). MiR-31 was significantly down-regulated in BC, t=38.45, p<0.0001.*

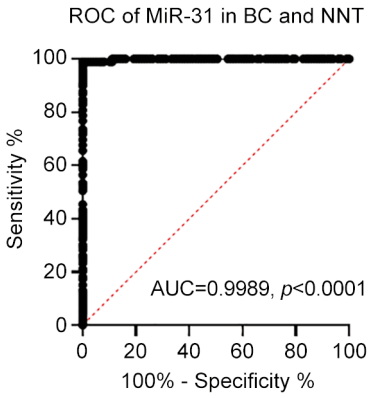


Figure 2. *ROC analysis of MiR-31 in breast cancer (BC) tissues and nearby normal tissues (NNT). MiR-31 has a high specificity for detection of malignancy in BC with area under the curve (AUC) of 0.9989 and p<0.0001.*

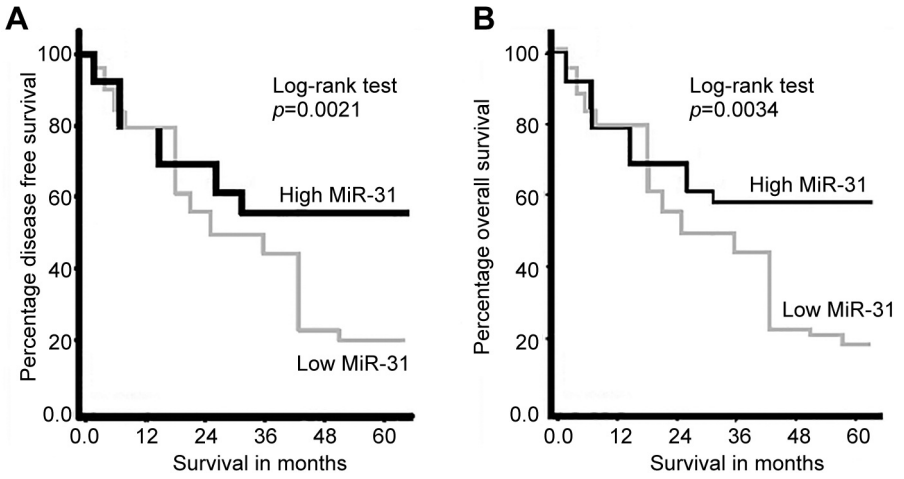


Figure 3. *Kaplan-Meier survival curves in patients with breast cancer according to MiR-31 expression. A) Patients with low MiR-31 expression had significantly shorter disease-free survival (p=0.0021). B) Low miR-expression correlated with short overall survival (p=0.0034).*

Meier survival curve was plotted to explore the relationship between miR-31 expression and survival of BC patients. Low miR-31 expression significantly corelated with short overall and disease-free survival, $p$=0.0021 and 0.0034, respectively (Figure 3).

## Discussion

MiR-31 was identified in HeLa cells and localized on chromosome 9p21.3 (17). Increasing data confirm that miR-31 has a varying pattern of expression in various cancers: it is over-expressed in colorectal carcinoma (18), squamous cell carcinoma of the head and neck (HNSCC) (19), HCC (8), and lung cancer (20). However, it is expressed at low levels in adenocarcinoma of the stomach (21), prostatic adenocarcinoma (22), transitional cell carcinoma of the urinary bladder (23), and serous adenocarcinoma of the ovary (24). Despite the ample reports about the variation in miR-31 expression patterns among malignant tumors, the molecular activities of miR-31 are still to be clarified, particularly in BC. A large amount of available data supports the principal impact of miRNAs in the control of metastasis, which directly impacts prognosis (25). The current research aimed to study the impact of miR-31 in BC biology and prognosis.

Our results revealed that miR-31 expression levels in BC tissues were significantly lower than those in NNT. Similar to our results, Stepicheva and Song (26) and Schmittgen

Table II. *The association of miR-31 expression with estrogen, progesterone, and HER2 receptor expression in breast cancer tissues.*

| Parameters | miR-31 | | | *p*-Value |
|---|---|---|---|---|
| | Number | Mean | SD | |
| Estrogen receptor (ER) | | | | |
| Positive | 74 | 1.23 | 0.90 | <0.0001[a] |
| Negative | 26 | 0.07 | 0.04 | |
| Progesterone receptor (PR) | | | | |
| Positive | 69 | 1.31 | 0.89 | <0.0001[a] |
| Negative | 31 | 0.10 | 0.08 | |
| HER2 receptor | | | | |
| Negative | 84 | 1.10 | 0.92 | <0.0001[a] |
| Positive | 16 | 0.05 | 0.03 | |

[a]Student's *t*-test.

(27), reported low expression levels of miR-31 in BC tissues. In contrast to our results, Lu *et al*. reported that there was no difference in miR-31 levels between malignant and non-malignant surrounding breast tissues (28).

Young age and premenopausal status are considered adverse prognostic features. In the present study, low expression levels of miR-31 were noted in younger premenopausal patients, in accordance to the report by Lu *et al*. (28). BC is classified into various subtypes, each with its own set of biological features (29). Our data revealed low expression levels of miR-31 in tumors was associated with poor prognosis such as infiltrative lobular carcinoma (ILC), and high-grade tumors. Additional poor prognostic features include: ER and PR negative tumors, Her2 positive tumors, large tumor size, metastases to axillary lymph nodes, and advanced TNM clinical stage. In addition, ER, PR, and HER2 receptor status are critical factors in the treatment decision-making of BC (29, 30). In our study, low expression levels of miR-31 were noted in ER and PR negative tumors, Her2 positive tumors, large primary tumors, in patients with extensive metastases to the axillary lymph nodes, and patients with advanced clinical stage. Similar results were reported by Stepicheva and Song (26) and Schmittgen (27). Lu *et al*. showed high levels of miR-31 in low grade tumors, early-stage tumors, infiltration to a limited number of axillary lymph nodes, and small primary tumors. In addition, miR-31 expression correlated with ER and PR expression but not with HER-2) expression (28). In our study, the association between extensive spread to axillary lymph nodes and low miR-31 expression could be explained considering miR-31 a critical regulator of BC metastasis through promoting cell invasiveness and aggressive BC (31). Other processes mediated by this miRNA have also been identified. There is strong evidence that Guanine nucleotide-binding protein subunit alpha-13 (GNA13) activation contributes to BC by promoting cell invasion through the regulation of post-transcriptional gene expression in breast cancer cell lines *via* miR-31. Therefore, down-regulation of miRNA-31 may promote cancer progression through an increase in GNA13 levels (32). Vimalraj *et al*. reported miR-31 as an onco-suppressor because it prevented BC cell metastasis at different phases by blocking prometastatic gene transcription (33). Furthermore, Korner *et al*. found that miR-31 up-regulation suppresses the carcinogenic NF-B axis, making it an onco-suppressor miRNA (34). In the current study, ROC curve analysis revealed that low levels of miR-31 predict BC with 100% specificity and 98% sensitivity, with AUC=0.9989. Therefore, it can be used as a diagnostic biomarker in BC. Lu *et al*. revealed that the levels of miR-31-5p differed considerably between oral cancer patients and healthy controls, as well as between pre- and post-operative patients (35). In addition, miR-31 has been reported to have diagnostic efficacy in lung cancer (36). Kaplan-Meier survival curve of miR-31 expression in BC patients revealed that patients with low miR-31 expression have significantly shorter overall and disease-free survival. These results are highly suggestive that low expression of miR-31 is significantly associated with adverse prognostic features in BC. In addition, miR-31 could be used as a marker to predict the prognosis and survival of BC patients; however, further studies are required, on a larger scale, to confirm these results.

## Conclusion

Our study revealed that miR-31 was significantly suppressed in BC. MiR-31 suppression was significantly linked to adverse clinical and pathological features, and short survival. MiR-31 could be used as a diagnostic and prognostic marker in BC.

## Conflicts of Interest

The Authors have no conflicts of interest to declare in relation to this study.

## Authors' Contributions

AFG and WHE conceptualized the study and contributed to the statistical analysis. EME and AEA contributed to the study design, data collection, and literature review. ASK, HJB, and AAS were involved in the laboratory work and wrote the initial draft. All Authors contributed to data interpretation, critically reviewed the manuscript, and approved the final version.

## Acknowledgements

The researchers gratefully thank the financial assistance of Taif University, this study was funded by Taif University Researchers Supporting Project number (TURSP-2020/255), Taif University, Taif, Kingdom of Saudi Arabia.

## References


1 Dolatkhah R, Somi MH, Jafarabadi MA, Hosseinalifam M, Sepahi S, Belalzadeh M, Nezamdoust M and Dastgiri S: Breast cancer survival and incidence: 10 years cancer registry data in the Northwest, Iran. Int J Breast Cancer *2020*: 1963814, 2020. PMID: 32411480. DOI: 10.1155/2020/1963814

2 Parker JS, Mullins M, Cheang MC, Leung S, Voduc D, Vickery T, Davies S, Fauron C, He X, Hu Z, Quackenbush JF, Stijleman IJ, Palazzo J, Marron JS, Nobel AB, Mardis E, Nielsen TO, Ellis MJ, Perou CM and Bernard PS: Supervised risk predictor of breast cancer based on intrinsic subtypes. J Clin Oncol *27(8)*: 1160-1167, 2009. PMID: 19204204. DOI: 10.1200/JCO.2008.18.1370

3 Beckmann MW, Niederacher D, Schnürch HG, Gusterson BA and Bender HG: Multistep carcinogenesis of breast cancer and tumour heterogeneity. J Mol Med (Berl) *75(6)*: 429-439, 1997. PMID: 9231883. DOI: 10.1007/s001090050128

4 Zamore PD and Haley B: Ribo-gnome: the big world of small RNAs. Science *309(5740)*: 1519-1524, 2005. PMID: 16141061. DOI: 10.1126/science.1111444

5 Abolghasemi M, Tehrani SS, Yousefi T, Karimian A, Mahmoodpoor A, Ghamari A, Jadidi-Niaragh F, Yousefi M, Kafil HS, Bastami M, Edalati M, Eyvazi S, Naghizadeh M, Targhazeh N, Yousefi B, Safa A, Majidinia M and Rameshknia V: MicroRNAs in breast cancer: Roles, functions, and mechanism of actions. J Cell Physiol *235(6)*: 5008-5029, 2020. PMID: 31724738. DOI: 10.1002/jcp.29396

6 Loh HY, Norman BP, Lai KS, Rahman NMANA, Alitheen NBM and Osman MA: The regulatory role of microRNAs in breast cancer. Int J Mol Sci *20(19)*: 4940, 2019. PMID: 31590453. DOI: 10.3390/ijms20194940

7 Laurila EM and Kallioniemi A: The diverse role of miR-31 in regulating cancer associated phenotypes. Genes Chromosomes Cancer *52(12)*: 1103-1113, 2013. PMID: 23999990. DOI: 10.1002/gcc.22107

8 Stepicheva NA and Song JL: Function and regulation of microRNA-31 in development and disease. Mol Reprod Dev *83(8)*: 654-674, 2016. PMID: 27405090. DOI: 10.1002/mrd.22678

9 Li Y, Quan J, Chen F, Pan X, Zhuang C, Xiong T, Zhuang C, Li J, Huang X, Ye J, Zhang F, Zhang Z and Gui Y: MiR-31-5p acts as a tumor suppressor in renal cell carcinoma by targeting cyclin-dependent kinase 1 (CDK1). Biomed Pharmacother *111*: 517-526, 2019. PMID: 30597305. DOI: 10.1016/j.biopha.2018.12.102

10 Kiss I, Mlcochova J, Bortlicek Z, Poprach A, Drabek J, Vychytilova-Faltejskova P, Svoboda M, Buchler T, Batko S, Ryska A, Hajduch M and Slaby O: Efficacy and toxicity of panitumumab after progression on cetuximab and predictive value of MiR-31-5p in metastatic wild-type KRAS colorectal cancer patients. Anticancer Res *36(9)*: 4955-4959, 2016. PMID: 27630355. DOI: 10.21873/anticanres.11063

11 Chi XJ, Wei LL, Bu Q, Mo N, Chen XY, Lan D and Zhou QN: Identification of high expression profiles of miR-31-5p and its vital role in lung squamous cell carcinoma: a survey based on qRT-PCR and bioinformatics analysis. Transl Cancer Res *8(3)*: 788-801, 2019. PMID: 35116817. DOI: 10.21037/tcr.2019.04.21

12 Schmittgen TD: miR-31: a master regulator of metastasis? Future Oncol *6(1)*: 17-20, 2010. PMID: 20021205. DOI: 10.2217/fon.09.150

13 O'Day E and Lal A: MicroRNAs and their target gene networks in breast cancer. Breast Cancer Res *12(2)*: 201, 2010. PMID: 20346098. DOI: 10.1186/bcr2484

14 Amin MB, Greene FL, Edge SB, Compton CC, Gershenwald JE, Brookland RK, Meyer L, Gress DM, Byrd DR and Winchester DP: The Eighth Edition AJCC Cancer Staging Manual: Continuing to build a bridge from a population-based to a more "personalized" approach to cancer staging. CA Cancer J Clin *67(2)*: 93-99, 2017. PMID: 28094848. DOI: 10.3322/caac.21388

15 Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. Methods *25(4)*: 402-408, 2001. PMID: 11846609. DOI: 10.1006/meth.2001.1262

16 Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, Perou CM, Regan MM, Rimm DL, Symmans WF, Torlakovic EE, Varella L, Viale G, Weisberg TF, McShane LM and Wolff AC: Estrogen and progesterone receptor testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists guideline update. Arch Pathol Lab Med *144(5)*: 545-563, 2020. PMID: 31928354. DOI: 10.5858/arpa.2019-0904-SA

17 Lagos-Quintana M, Rauhut R, Lendeckel W and Tuschl T: Identification of novel genes coding for small expressed RNAs. Science *294(5543)*: 853-858, 2001. PMID: 11679670. DOI: 10.1126/science.1064921

18 Eneh S, Heikkinen S, Hartikainen JM, Kuopio T, Mecklin JP, Kosma VM and Mannermaa A: MicroRNAs associated with biological pathways of left- and right-sided colorectal cancer. Anticancer Res *40(7)*: 3713-3722, 2020. PMID: 32620610. DOI: 10.21873/anticanres.14360

19 Rich JN: Cancer stem cells in radiation resistance. Cancer Res *67(19)*: 8980-8984, 2007. PMID: 17908997. DOI: 10.1158/0008-5472.CAN-07-0895

20 Liu X, Sempere LF, Ouyang H, Memoli VA, Andrew AS, Luo Y, Demidenko E, Korc M, Shi W, Preis M, Dragnev KH, Li H, Direnzo J, Bak M, Freemantle SJ, Kauppinen S and Dmitrovsky E: MicroRNA-31 functions as an oncogenic microRNA in mouse and human lung cancer cells by repressing specific tumor suppressors. J Clin Invest *120(4)*: 1298-1309, 2010. PMID: 20237410. DOI: 10.1172/JCI39566

21 Zhang Y, Guo J, Li D, Xiao B, Miao Y, Jiang Z and Zhuo H: Down-regulation of miR-31 expression in gastric cancer tissues and its clinical significance. Med Oncol *27(3)*: 685-689, 2010. PMID: 19598010. DOI: 10.1007/s12032-009-9269-x

22 Schaefer A, Jung M, Mollenkopf HJ, Wagner I, Stephan C, Jentzmik F, Miller K, Lein M, Kristiansen G and Jung K: Diagnostic and prognostic implications of microRNA profiling in prostate carcinoma. Int J Cancer *126(5)*: 1166-1176, 2010. PMID: 19676045. DOI: 10.1002/ijc.24827

23 Wszolek MF, Rieger-Christ KM, Kenney PA, Gould JJ, Silva Neto B, Lavoie AK, Logvinenko T, Libertino JA and Summerhayes IC: A MicroRNA expression profile defining the invasive bladder tumor phenotype. Urol Oncol *29(6)*: 794-801.e1, 2011. PMID: 19945312. DOI: 10.1016/j.urolonc.2009.08.024

24 Creighton CJ, Fountain MD, Yu Z, Nagaraja AK, Zhu H, Khan M, Olokpa E, Zariff A, Gunaratne PH, Matzuk MM and Anderson ML: Molecular profiling uncovers a p53-associated role for microRNA-31 in inhibiting the proliferation of serous

ovarian carcinomas and other cancers. Cancer Res *70(5)*: 1906-1915, 2010. PMID: 20179198. DOI: 10.1158/0008-5472.CAN-09-3875

25 Soheilyfar S, Velashjerdi Z, Sayed Hajizadeh Y, Fathi Maroufi N, Amini Z, Khorrami A, Haj Azimian S, Isazadeh A, Taefehshokr S and Taefehshokr N: *In vivo* and *in vitro* impact of miR-31 and miR-143 on the suppression of metastasis and invasion in breast cancer. J BUON *23(5)*: 1290-1296, 2018. PMID: 30570849.

26 Stepicheva NA and Song JL: Function and regulation of microRNA-31 in development and disease. Mol Reprod Dev *83(8)*: 654-674, 2016. PMID: 27405090. DOI: 10.1002/mrd.22678

27 Schmittgen TD: miR-31: a master regulator of metastasis? Future Oncol *6(1)*: 17-20, 2010. PMID: 20021205. DOI: 10.2217/fon.09.150

28 Lu Z, Ye Y, Jiao D, Qiao J, Cui S and Liu Z: miR-155 and miR-31 are differentially expressed in breast cancer patients and are correlated with the estrogen receptor and progesterone receptor status. Oncol Lett *4(5)*: 1027-1032, 2012. PMID: 23162645. DOI: 10.3892/ol.2012.841

29 Al-Saleh K, Salah T, Arafah M, Husain S, Al-Rikabi A and Abd El-Aziz N: Prognostic significance of estrogen, progesterone and HER2 receptors' status conversion following neoadjuvant chemotherapy in patients with locally advanced breast cancer: Results from a tertiary Cancer Center in Saudi Arabia. PLoS One *16(3)*: e0247802, 2021. PMID: 33667252. DOI: 10.1371/journal.pone.0247802

30 Inubushi S, Kawaguchi H, Mizumoto S, Kunihisa T, Baba M, Kitayama Y, Takeuchi T, Hoffman RM, Tanino H and Sasaki R: Oncogenic miRNAs identified in tear exosomes from metastatic breast cancer patients. Anticancer Res *40(6)*: 3091-3096, 2020. PMID: 32487603. DOI: 10.21873/anticanres.14290

31 Powrózek T and Małecka-Massalska T: Is microRNA-31 a key regulator of breast tumorigenesis? J Thorac Dis *10(2)*: 564-566, 2018. PMID: 29608199. DOI: 10.21037/jtd.2017.12.133

32 Rasheed SA, Teo CR, Beillard EJ, Voorhoeve PM, Zhou W, Ghosh S and Casey PJ: MicroRNA-31 controls G protein alpha-13 (GNA13) expression and cell invasion in breast cancer cells. Mol Cancer *14*: 67, 2015. PMID: 25889182. DOI: 10.1186/s12943-015-0337-x

33 Vimalraj S, Miranda PJ, Ramyakrishna B and Selvamurugan N: Regulation of breast cancer and bone metastasis by microRNAs. Dis Markers *35(5)*: 369-387, 2013. PMID: 24191129. DOI: 10.1155/2013/451248

34 Körner C, Keklikoglou I, Bender C, Wörner A, Münstermann E and Wiemann S: MicroRNA-31 sensitizes human breast cells to apoptosis by direct targeting of protein kinase C epsilon (PKCepsilon). J Biol Chem *288(12)*: 8750-8761, 2013. PMID: 23364795. DOI: 10.1074/jbc.M112.414128

35 Lu Z, He Q, Liang J, Li W, Su Q, Chen Z, Wan Q, Zhou X, Cao L, Sun J, Wu Y, Liu L, Wu X, Hou J, Lian K and Wang A: miR-31-5p is a potential circulating biomarker and therapeutic target for oral cancer. Mol Ther Nucleic Acids *16*: 471-480, 2019. PMID: 31051332. DOI: 10.1016/j.omtn.2019.03.012

36 Wang S, Wang Z, Wang Q, Cui Y and Luo S: Clinical significance of the expression of miRNA-21, miRNA-31 and miRNA-let7 in patients with lung cancer. Saudi J Biol Sci *26(4)*: 777-781, 2019. PMID: 31049003. DOI: 10.1016/j.sjbs.2018.12.009

*Received February 2, 2022*
*Revised March 11, 2022*
*Accepted March 14, 2022*