Research Square

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Expression of genes related to iron homeostasis in breast cancer

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Research Article

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Abstract

Background: The dysfunctions in the metabolism of iron has an important role in many pathological conditions, ranging from disease with iron deposition to cancer. Studies on malignant diseases of the breast reported irregular expression in genes associated with iron metabolism. The variations are related with findings which have prognostic significance. This study evaluated the relationship of the expression levels of *transferrin receptor 1* (*TFRC), iron regulatory protein 1* (*IRP1*), *hepcidin* (*HAMP*), *ferroportin 1* (*FPN1*), *hemojuvelin* (*HFE2), matriptase 2* (*TMPRSS6*), and *miR-122* genes in the normal and malignant tissues of breast cancer patients.

Methods & Results: The normal and malignant tissues from 75 women with breast malignancies were used in this study. The patients did not receive any treatment previously. Reverse transcription-quantitative polymerase chain reaction (RT-qPCR) was used in figuring the levels of gene expression associated with the iron metabolism. When the malignant and normal tissues gene expression levels were analyzed, expression of *TFRC* increased (1.586-fold); *IRP1* (0.594 fold) and *miR-122* (0.320 fold) expression decreased; *HAMP*, *FPN1*, *HFE2* and *TMPRSS6* expressions did not change. *FPN1* and *IRP1* had a positive association, and this association was statistically significant (r=0.266; p=0.022). *IRP1* and *miR-122* had a positive association, and this association had statistical significance (r=0.231; p=0.048)

Conclusions: Our study portrayed the important association among genes involved in iron hemostasis and breast malignancy. The results could be used to establish new diagnostic techniques in the management of breast malignancies. The alterations in the metabolism of malignant breast cells with normal breast cells could be utilized to achieve advantages in treatment.

Introduction

Malignancies of the breast cancer account for 12.5% of the newly diagnosed malignancies, globally, making it the most commonly seen cancer [1]. The etiology of breast cancer is multifactorial. Lifestyle factors, environmental factors and genetical factors are related with the malignant transformation [2].

Iron has major functions in the metabolism in many organisms. However, the exuberance of iron could lead to cellular injury by the free radicals. Disorders of the iron metabolism are associated with many diseases and malignant transformation. Cellular proliferation and tumorigenesis is linked with iron metabolism. Bogdan et al. showed the elevation in cellular iron levels in malignant tissues, caused by disruption of metabolism of iron [3]. Silva B. et al showed the presence of various mechanisms in iron homeostasis and the relationship between these mechanisms have major significance [4].

The homeostasis of iron is maintained controlling of its absorption, mobilization of the iron storage and recycleing. Iron is transported in the blood by transferrin. It has transferrin receptor protein 1 (TfR1) on its surface of cell, which is a specific receptor. TfR1 is coded by by the *transferrin receptor 1* (*TFRC*) gene. Cytoplasmic proteins, Iron regulatory proteins 1 & 2 (IRP-1 & IRP-2) [5], also called cellular "iron sensor proteins", control the translation of genes responsible for the iron and energy metabolism. The decrease

in the intracellular iron levels lead to the activation of IRP1, which binds to TfR mRNA and its stabilization, leading to the decrease of the translation. Increases in the levels of the iron in the cell lead to the inhibition of the iron responsive elements (IRE)-binding capacity of IRP1 [6].

Hepcidin is a peptide hormone, formed by 25 amino acids. *Hepcidin antimicrobial peptide* (*HAMP*) gene has the genetic code for Hepcidin. It is secreted by the hepatocytes and it plays a role in the tissue distribution and absorption of iron [7]. Iron absorption in the intestinal tract, plasma iron concentrations, distribution of iron to the tissues are controlled by hepcidin. This process start by the initiation of the deterioration of the receptor. Ferroportin (*FPN1*; *ferroportin-1* or *SLC40A1 gene*) has major part in the transportation of iron between cells and blood. The linking of hepcidin to ferroportin starts its internalization, consequently its degradation; giving was to the increase in the levels of iron intracellularly [8].

Hemojuvelin (HJV) is one of the main components of the BMP-SMAD signaling pathway. The rise of iron stores triggers hepcidin expression upregulation [9]. *Hemochromatosis type 2 protein* (*HFE2*) gene encode HJV. Matriptase 2 is encoded by the *Transmembrane serine protease 6* (*TMPRSS6*) gene. BMP co-receptor HJV which is located in the BMP-SMAD pathway, is cleaved by the Matriptase 2. This helps control the expression of hepcidin. miRNAs, especially miR-122, has been reported to control hemojuvelin levels [9]. miR-122 acts on mRNA expression of hemojuvelin, in the liver tissue, which is a major part in controlling the tissue iron levels [10].

This study aimed to compare the levels of expression of *TFRC, IRP1, HAMP, FPN1, HFE2, TMPRSS6*, and *miR-122* genes in the normal and malignant tissues of patients with breast cancer.

Materials And Methods

Seventy-five female patients with primary breast cancer who were operated before any form of neoadjuvant treatment were involved in this study. The mean age was 54 (± 13 years). The inclusion criteria were the presence of primary breast malignancy. The exclusion criteria were, administration of any neoadjuvant treatment (radiotherapy and/or chemotherapy and/or endocrine therapy) presence of other cancers, recurrent breast cancer, unavailability of clinical data. Istanbul University-Cerrahpasa Cerrahpasa Medical School Institutional Review Board approved the study. It was performed based on the principles of the Declaration of Helsinki. Informed consent was acquired from all of participants.

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)

The tissue samples were taken from the tumor and the normal tissues outside the tumor microenvironment. PureLink® RNA Mini Kit (Thermo Fisher Scientific, USA) was utilized for the isolation of the total RNA. NanoDrop Spectrophotometer (Thermo Fisher Scientific, USA) was used to find the levels of the total RNA. The level of the total RNA calculation was used for the equalization for the initial RNA used for cDNA synthesis. TranScriba Kit (A&A Biotechnology, Poland) was utilized for the cDNA synthesis. StepOnePlus Real-Time PCR System appliance (Applied Biosystems, USA) was utilized for

qPCR analyses. TaqMan® Gene Expression Master Mix (Thermo Fisher Scientific, USA) and Taqman® Gene Expression Assays (Thermo Fisher Scientific, USA) were used for the qPCR analysis. The recommendations from the manufactorer were followed. The process started with 50°C at two minutes. The initial denaturation-hot start at 95°C (Holding Stage) for 10 minutes, accompanied by 40 cycles of the two-step reaction (Cycling Stage). The two step reaction was 10 seconds at 95°C and sixty seconds at 60°C. The expression studies for the genes were done in triplicate.

Statistical Analysis

Quantitative-Comparative Ct (ΔΔCт) method was used for finding the gene expression levels. $2^{-\Delta\Delta C_T}$ values of the target genes were calculated by Cycle Threshold (CT) values of Actin, beta gene (*ACTB)*. ACTB gene was used as a housekeeping gene. *miR-122*'s relative expression was determined by the utilization of *RNU44* as the housekeeping gene. the $2^{-\Delta\Delta C_T}$method was used to observe the relative changes in the target and the housekeeping gene expression in the normal tissue samples and the malignant tissue samples [11]. Mean+standard deviation (SD) was used for the age or as mean+error (SE) was used for the gene expression; in the expression of the continuous values. $p<0.05$ was accepted as the statistical significance level. Mann-Whitney test was used for the analysis of the two groups in RT-qPCR, for statistical analysis. The relationship between gene expressions was analyzed by the utilization of Pearson correlation . The statistical analysis was done by statistical software package (SPSS, v18.0, SPSS Inc., Chicago, IL).

Results

In this study, the expression levels of *TFRC, IRP1, HAMP, FPN1, HFE2, TMPRSS6* and *miR-122* genes in the malignant tissues and the normal tissue samples near the malignant tissues from 75 female patients were determined (Table 3).

The expression levels of *TFRC* in the malignant tissues were 1.586 fold increased in comparison with the normal tissues based on $2^{-\Delta\Delta C_T}$ calculations. The expression levels of *TFRC* in the malignant tissues (0.0115± 0.0012) were increased when compared with the normal tissue expression levels of *TFRC* (0.0062± 0.0005)($p=0.0001$). On the other hand, the expression levels of *IRP1* in the malignant tissue was found to be 0.594 fold decreased compared to the normal tissue. The expression levels of *IRP1* in the malignant tissue (0.0034± 0.0003) were lower than the expression levels of *IRP1* in thenormal tissue (0.0055± 0.0004); and this difference was statistically significant ($p = 0.0001$). Also, the *miR-122* expression levels in tumor tissue were found to be 0.32 fold decreased when compared to the normal tissue. The expression levels of *miR-122* in the malignant tissue (0.0003± 0.0002) were 0.32 fold decreased than its expression levels in the normal tissue (0.0008± 0.0001). This difference had statistical significance ($p = 0.025$).

The expression levels of *TMPRSS6* in the malignant tissues (0.0190± 0.0121) were 1.912 fold increased than the normal tissue (0.0133± 0.0029). Nonetheless, the difference was not statistically

significant (p=0.651). The expression levels of *HFE2* in the malignant tissues (0.0228± 0.0080) was 0.531 fold decreased than the expression levels in the normal tissue(0.0312± 0.0050). The diffrence did not reach statistical significance (p = 0.368). The expression levels of *HAMP* in the malignant tissue (0.0057±0.0012) was 1.34 fold increased than the expression levels in the normal tissue(0.0055±0.0011), however the difference was not statistically significant (p = 0.911). The *FPN1* expression levels in the tumor tissue were found to be 0.627 fold decreased when compared to the normal tissue. The expression levels of *FPN1* in the malignant tissue (0.0319± 0.0051) was 0.627 fold decreased than the expression levels in the normal tissue (0.0331± 0.0028); and the difference did not reach statistical significance (p = 0.836).

According to the calculation of $2^{-\Delta\Delta C_T}$, 0.9-1.1 range was used for the evaluation. When the value was lower than 0.9, the gene expression was accepted to be less than the expression in the normal tissue. 0.9–1.1 range was accepted as not changed in comparison with the expression in the normal tissue. 1.1 and values above this level was defined as increased gene expresion in comparison with the normal tissue. *TFRC* gene expression was found to be elevated in 50 (67%), reduced in 17 (23%) tissues. The *TFRC* gene expression was not altered in 8 (10%) tissues. IRP1 gene expression was elevated in 8(11%), reduced in 58 (77%) tissues; and it was not altered in 9 (12%) tissues. The *miR-122* gene expression was elevated in 12 (16%), reduced in 62 (83%) tissues; and not altered in 1 (%1) tissues. The *TMPRSS6* gene expression elevated in 46 (61%), reduced in 26 (35%) tissues and it was not altered in 3 (4%) tissues. The *HFE2* gene expression was elevated in 16 (21%), reduced in 50 (67%) tissues and it was not altered in 9 (12%) tissues. The *HAMP* gene expression was elevated in 35 (47%), reduced in 30 (40%) tissues, and it was not altered in 10 (13%) tissues. The *FPN1* gene expression was elevated in 27 (36%), reduced in 42 (56%) tissues; and it was not altered in 6 (8%) tissues.

In our study, the relationship between *TFRC, IRP1, HAMP, FPN1, HFE2, TMPRSS6* and *miR122* expression levels was evaluated using the Pearson correlation test. Two statistically significant relationships were unraveled. These were *IRP1* and *FPN1* (r=0.266; p=0.022), and *IRP1* and *miR-122* (r=0.231; p=0.048).

Discussion

Increased levels of cellular iron is vital for growth and rapid proliferation of the cancer cells. Various studies portrayed the importance of dysregulation of proteins enrolled in iron metabolism, have vital parts in malignant transformation. Alterations of genes related with iron hemostatsis had been thought to be prognostic biomarkers and therapeutic targets in malignancies. Miller et al.'s study reported that attrition of genes related with iron excrition and in addition to this the upregulation of iron uptake process had effects on the prognosis of breast cancer [12].

The *TFRC* gene encodes TfR1, a receptor located in cell surface, esential for the iron absorption into the cell. TfR1 had increased expression in cells with rapid proliferation, like cancer cells, which have high demang of iron during the proliferaiton. Based on this knowledge, the upregulation of the *TFRC* gene had been defined as a target candidate in cancer therapy. Increased expression of this gene was found to be

associtaed with cancer cell proliferation and poor survival in patients with breast malignancy, in a report by Habashy et al [13]. Jiang et al. reported the expression of *TFRC* was elevated in malignant breast cells (MCF-7 cells) than cells in the normal mammary glands(MCF-12A cells) [14]. In allignment with these prior findings, in our study the expression levels of TFTC was significantly upregulated in the malignant breast cells.

Transcriptional, post-transcriptional processes effect the TFRC expression. The iron responsive element system (IRP1/2-IRE), controls the iron homeostasis through post-transcriptional modulation of the TFRC through a sophisticated system. Chen et al. showed that deprevation of iron led to the binding of IRP1/2 to IREs, which led to the stabilization of the mRNA of TFRC, thus raising the expression of proteins [15]. Wang et al. showed that induced *IRP1* expression in malignant breast cells (MCF7) resulted in elevation of mRNA and protein levels of TFRC [16]. The expression level of *IRP1* gene was reduced in the malignant cells in our study. Therefore, we can not postulate the relationship among the elevation of the expression of *TFRC* and expression levels of *IRP1* gene. Wang et al. showed that overexpression of *IRP2*, was associated with reduced ferritin heavy chain and TfR1 elevation. However this relationship was not present for *IRP1*. Wang et al, reported that *IRP2* expression alone was associated with the molecular subtype and the histological grade of the human breast cancer [16]. Increased levels of TfR1 expression were reported in prostate cancer cells [17]. In a study by Deng et al., the expression level of *IRP2* was detected in 4 malignant prostate cell lines, *IRP1* expression level had moderate risein only 2 cell lines [17]. The increase in the regulation of *IRP2* in malignant prostate cells homogenizes normal iron control pathways, which gives way to elevation in the levels of iron, aiding tumor proliferation. *IRP1* was reported to be downregulation in hepatocellular carcinoma and this could be used to predict the stage of the tumor and the prognosis [18]. Oxygen, oxidative stress and the levels of nitric oxide are among the additional molecules and processes associated with the respond of IRPs in addition to cellular iron levels. It was reported by Luo et al. that hypoxia in the short term lead to a decline in mRNA of IRP1and levels of protein in HepG2 cells [19]

The most essential iron efflux transporter is ferroportin protein (FPN1). Its function is the siphoneage of the iron to the cytoplasm of the macrophages and the enterocytes. FPN1 is encoded by the *SLC40A1* gene. Its expression if controlled by hepcidin, encoded by the *HAMP* gene [20]. The dysregulated hepcidin–FPN1 signaling was related with rise in malignant transformation by experimental and epidemiological studies.. However, the exact processes associated with the dysregulation of iron-related gene expression in the malignant processes are still not uncovered. In our study, no difference in the hepcidin gene expression was identified. On the other hand, a statistically insignificant decrease in the *FPN*1 gene expression was seen. The rise in hepcidin leves were seen in the lung, breast, prostate, renal cancer in addition to various other cancers. However, its levels were reduced in some brain tumors and hepatocellular carcinoma [21]. Shen et al. reported the findings in hepatocellular carcinoma, where the levels of hepcidin was reduced in malignant cells than the normal cells [22]. In breast cancer, Zhang et al. reported limited elevations in the expression levels of hepcidin in malignant cells than the normal tissues [23]. Similar to our study, the tumor ferroportin concentration was found to be low in the breast tumors in that study. In addion, Pinnix et al.'s study revealed that the mRNA level of tumor hepcidin was

able to discern only marginal significance for patient prognosis [24]. The major and most prominent hepcidin expression simulators are bone morphogenetic proteins (BMPs).. Reports have shown that BMP6 was not alone, other BMPs also regulate hepcidin expression [21]. The UniGene database's in silico analysis showed that, malignant breast tissue had reduced expression of ferroportin [25]. Our study showed that ferroportin gene expression in the cancer tissue was also found to be 0.627 times reduced compared to the normal tissue.

The coreceptor of BMP, hemojuvelin's clevage blocks the BMP-SMAD signaling pathway, through inhibition of the expression of hepcidin by matriptase-2 [26]. In our study, matriptase-2 expression increased (1,912 fold) and hemojuvelin gene expression decreased (0.531 fold) in the malignant breast tissues. In our previous study that we conducted in breast cancer patients, the levels of the expression of *TMPRSS6* gene in the malignant tissues were 1.88 fold elevated than normal tissues [27]. Normal breast tissue samples was reported to have dominant matriptase-2 expression [28]. Overall et al.'s study showed that the gene expression levels of *TMPRSS6* was elevated in the invasive ductal carcinoma cells [29]. Gitlin-Domagalska et al. showed the relationship of *TMPRSS6* with decline in gene expression levels, progression of tumor cell, and poor prognosis [30].

We could not find any literature investigating homojuvenile gene expression in breast cancer. The simulation of hepcidin transcription by BMP is inhibitted by matriptase-2. This process is achieved by the cell surface proteolytic processing of the BMP co-receptor hemojuvelin [28]. Controversial results had been reported on BMPs role in malignancies of the breast. The BMP6's role in breast malignancies had been found to be variable according to several studies, performed on the relationship between hemojuvelin and BMP6. Most of the reports present that BMP6 expression is reduced in the malignant breast tissue [31]. Our study showed that the gene expression of *TMPRSS6* elevated in the malignant tissues. An hypothesis could be constructed on the interaction between matriptase-2 with the BMP6/SMAD signaling via by hemojuvelin. The low homojuvenile expression we found in our study supported this hypothesis.

Hepatic *miR-122* expression had paramount importance in sustaining the iron levels in the liver andthe plasma. Castoldi et al. reported that the inhibition of the expression of hepatic *miR-122* in the liver tissue of mice and in primary murine hepatocytes, lead to a rise of mRNA expression of hepsidine and homojuvenile [10]. We portrayed that of *miR-122* expression was reduced in the malignant breast tissues. On the other hand, in our study, we found a decrease in expression of homojuvenile in malignant breast tissues, while the hepcidin expression was unchanged. miR-122 was reported to be essential in the regulation of serum cholesterol levels [32]. Therefore, the overlapping or non-overlapping metabolic pathways in which miR-122 plays a role may alter the effects of mir-122 on gene expressions. On the other hand, various studies studied the role of mir-122 in malignant breast tissue. Similar to our study, Yan et al reported that *miR-122* levels were reduced decreased in malignant breast cells than the normal tissue samples; and this finding was statistically significant [33]. Two studies portrayed the *miR-122* downregulation in malignant breast cells [34, 35]. Several other studies showed that expression of *miR-122* elevated in malignant breast tissue than the normal tissue [36].

In conclusion, our study shows a powerful associatin between genes enrolled in the metabolism of iron and the breast cancer, paving the way for the establishment of new tools to be used for the prognosis of breast cancer. They could also aid in the establishment to find new methods to identify normal and malignant breast cells, which could be utilized for to therapeutic advantage.

Declarations

COMPLIANCE WITH ETHICAL STANDARDS

- Funding: Scientific Research Project Coordination Unit of Istanbul University-Cerrahpasa funded the research, Project No. 22092

- The authors declare no conflict of interest.
- The ethical approval was obtained from Istanbul University-Cerrahpasa Cerrahpasa Medical School Institutional Review Board on June 6th, 2016 with the serial code of 214246. All of the procedures on human participants were in accordance with the declaration of Helsinki in 1964, its ammendments and parallel ethical standards.
- All the patients in this study provided informed consent before inclusion.
- The data of the study are available upon request from the corresponding author.

References

1. Siegel RL, Miller KD, Jemal A (2020) Cancer statistics. Cancer J Clin 70: 7–30. https://doi.org/10.3322/caac.21590.
2. Bogdanova N, Helbig S, Dörk T (2013) Hereditary breast cancer: ever more pieces to the polygenic puzzle. Hered Cancer Clin Pract 11: 12. https://doi: 10.1186/1897-4287-11-12
3. Bogdan AR, Miyazawa M, Hashimoto K, Tsuji Y (2016) Regulators of Iron Homeostasis: New Players in Metabolism, Cell Death, and Disease. Trends Biochem Sci. 41:274-286. https://doi: 10.1016/j.tibs.2015.11.012.
4. Silva B, Faustino P (2015) An overview of molecular basis of iron metabolism regulation and the associated pathologies. Biochim Biophys Acta1852:1347-1359. https://doi: 10.1016/j.bbadis.2015.03.011
5. Huang N, Zhan LL, Cheng Y, Wang XL, Wei YX, Wang Q, Li WJ (2020) TfR1 Extensively Regulates the Expression of Genes Associated with Ion Transport and Immunity. Curr Med Sci 40:493-501. https://doi: 10.1007/s11596-020-2208-y
6. Sanchez M, Galy B, Schwanhaeusser B, Blake J, Bähr-Ivacevic T, Benes V, Selbach M, Muckenthaler MU, Hentze MW (2011) Iron regulatory protein-1 and -2: transcriptome-wide definition of binding mRNAs and shaping of the cellular proteome by iron regulatory proteins Blood 118: e168-79. https://doi: 10.1182/blood-2011-04-343541

7. Liang L, Liu H, Yue J, Liu LR, Han M, Luo LL, Zhao YL, Xiao H (2017) Association of Single-Nucleotide Polymorphism in the Hepcidin Promoter Gene with Susceptibility to Extrapulmonary Tuberculosis. Genet Test Mol Biomarkers. 21: 351-356. https://doi: 10.1089/gtmb.2016.0300.
8. Ganz T, Nemeth E (2012) Hepcidin and iron homeostasis Biochim Biophys Acta 1823:1434-1443. https://doi: 10.1016/j.bbamcr.2012.01.014
9. Core AB, Canali S, Babitt JL (2014) Hemojuvelin and bone morphogenetic protein (BMP) signaling in iron homeostasis Front Pharmacol 5:104. https://doi: 10.3389/fphar.2014.00104
10. Castoldi M, Vujic Spasic M, Altamura S, Elmén J, Lindow M, Kiss J, Stolte J, Sparla R, D'Alessandro LA, Klingmüller U, Fleming RE, Longerich T, Gröne HJ, Benes V, Kauppinen S, Hentze MW, Muckenthaler MU (2011). The liver-specific microRNA miR-122 controls systemic iron homeostasis in mice. J Clin Invest 121:1386-1396. . https://doi: 10.1172/JCI44883
11. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method. Methods 25:402–408. https ://doi.org/10.1006/meth.2001.1262.
12. Miller LD, Coffman LG, Chou JW, Black MA, Bergh J, D'Agostino R Jr, Torti SV, Torti FM (2011) An Iron Regulatory Gene Signature Predicts Outcome in Breast Cancer. Cancer Res71:6728-6737. https://doi: 10.1158/0008-5472.CAN-11-1870.
13. Habashy HO, Powe DG, Staka CM, Rakha EA, Ball G, Green AR, Aleskandarany M, Paish EC, Douglas Macmillan R, Nicholson RI, Ellis IO, Gee JM (2010) Transferrin receptor (CD71) is a marker of poor prognosis in breast cancer and can predict response to tamoxifen. Breast Cancer Res Treat 119:283–293. https://doi.org/10.1007/s10549-009-0345-x
14. Jiang XP, Elliott RL, Head JF (2010) Manipulation of iron transporter genes results in the suppression of human and mouse mammary adenocarcinomas. Anticancer Res 30: 759–65. PMID:20392994
15. Chen Fei, Yumei Fan, Jiajie Hou, Bing Liu, Bo Zhang, Yanan Shang, Yanzhong Chang, Pengxiu Cao, Ke Tan (2021) Integrated analysis identifies TfR1 as a prognostic biomarker which correlates with immune infiltration in breast cancer. Aging (Albany NY) 13: 21671–21699. doi: 10.18632/aging.203512
16. Wang J, Pantopoulos K (2002) Conditional derepression of ferritin synthesis in cells expressing a constitutive IRP1 mutant. Mol Cell Biol 22: 4638-51. https://doi:10.1128/MCB.22.13.4638-4651.2002.
17. Deng Z, Manz DH, Torti SV, Torti FM (2017) Iron-responsive element-binding protein 2 plays an essential role in regulating prostate cancer cell growth. Oncotarget 8:82231-82243. https://doi: 10.18632/oncotarget.19288
18. Xue X, Ramakrishnan SK, Weisz K, Triner D, Xie L, Attili D, Pant A, Gyor_yB, Zhan M, Carter-Su C (2016) Iron Uptake via DMT1 Integrates Cell Cycle with JAK-STAT3 Signaling to Promote Colorectal Tumorigenesis. Cell Metab 24:447–446. https://doi: 10.1016/j.cmet.2016.07.015
19. Luo QQ, Wang D, Yu MY, Zhu L (2011) Effect of hypoxia on the expression of iron regulatory proteins 1 and the mechanisms involved. IUBMB Life 63:120-128. https://doi: 10.1002/iub.419.

20. Aschemeyer S, Qiao B, Stefanova D, Valore EV, Sek AC, Ruwe TA, Vieth KR, Jung G, Casu C, Rivella S (2017) Structure-function analysis of ferroportin defines the binding site and an alternative mechanism of action of hepcidin. Blood 131: 899–910. https://doi: 10.1182/blood-2017-05-786590
21. Vela D, Vela-Gaxha Z (2018) Differential regulation of hepcidin in cancer and non-cancer tissues and its clinical implications. Exp Mol Med 50: e436. https:// doi: 10.1038/emm.2017.273
22. Shen Y, Li X, Su Y, Badshah SA, Zhang B, Xue Y, Shang P (2019) HAMP Downregulation Contributes to Aggressive Hepatocellular Carcinoma via Mechanism Mediated by Cyclin4-Dependent Kinase-1/STAT3 Pathway. Diagnostics (Basel). 9:48. https://doi: 10.3390/diagnostics9020048.
23. Zhang S, Chen Y, Guo W, Yuan L, Zhang D, Xu Y, Nemeth E, Ganz T, Liu S (2014) Disordered hepcidin–ferroportin signaling promotes breast cancer growth Cell Signal 26: 2539-2550. https://doi: 10.1016/j.cellsig.2014.07.029
24. Pinnix ZK, Miller LD, Wang W, D'Agostino R Jr, Kute T, Willingham MC, Hatcher H, Tesfay L, Sui G, Di X, Torti SV, Torti FM (2010) Ferroportin and iron regulation in breast cancer progression and prognosis. Sci Transl Med 2:43ra56. https://doi:10.1126/scitranslmed.3001127
25. Chen S, Zhu B, Yu L (2006). In silico comparison of gene expression levels in ten human tumor types reveals candidate genes associated with carcinogenesis. Cytogenet Genome Res 112:53–59. https://doi: 10.1159/000087513
26. Maxson JE, Chen J, Enns CA, Zhang AS (2010) Matriptase-2-and proprotein convertase-cleaved forms of hemojuvelin have different roles in the downregulationof hepcidin expression. J Biol Chem 285:39021–39028. https://doi:10.1074/jbc.M110.183160
27. Mete M, Trabulus DC, Talu CK, Ozoran E, Mutlu T, Tekin B, Guven M (2020) An investigation of the relationship between TMPRSS6 gene expression, genetic variants and clinical findings in breast cancer. Mol Biol Rep 47:4225-4231. https://doi:10.1007/s11033-020-05498-0
28. Parr C, Sanders AJ, Davies G, Martin T, Lane J, Mason MD, Mansel RE, Jiang WG (2007) Matriptase-2 inhibits breast tumor growth and invasion and correlates with favorable prognosis for breast cancer patients. Clin Cancer Res 13:3568-5576. https://doi.org/10.1158/1078-0432.CCR-06-2357
29. Overall CM, Tam EM, Kappelhoff R, Connor A, Ewart T, Morrison CJ (2004) Protease degradomics: mass spectrometry discovery of protease substrates and the CLIP-CHIP, a dedicated DNA microarray of all human proteases and inhibitors. Biol Chem 385:493–504. https://doi.org/10.1515/BC.2004.058
30. Gitlin-Domagalska A, Mangold M, Dębowski D, Ptaszyńska N, Łęgowska A, Gütschow M, Rolka K (2018) Matriptase-2: monitoring and inhibiting its proteolytic activity. Future Med Chem 10:1–8. . https://doi.org/10.4155/fmc-2018-0346
31. Clement JH, Sänger J, Höffken K (1999) Expression of bone morphogenetic protein 6 in normal mammary tissue and breast cancer cell lines and its regulation by epidermal growth factor. Int J Cancer 80:250–256. https://doi:10.1002/(sici)1097-0215(19990118)80:2<250::aid-ijc14>3.0.co;2-d.
32. Feinberg MW, Moore KJ (2016) MicroRNA regulation of atherosclerosis. Circ Res 118:703-720. doi: 10.1161/CIRCRESAHA.115.306300.

33. Yan Y, Zhang F, Fan Q, Li X, Zhou K (2014) Breast cancer-specific TRAIL expression mediated by miRNA response elements of let-7 and miR-122. Neoplasma. 61: 672-9. doi: 10.4149/neo_2014_082.
34. Wang B, Wang H, Yang Z (2012) MiR-122 inhibits cell proliferation and tumorigenesis of breast cancer by targeting IGF1R. PLoS One.7:e47053. doi: 10.1371/journal.pone.0047053.
35. Ergün S, Ulasli M, Igci YZ, Igci M, Kırkbes S, Borazan E, Balik A, Yumrutaş Ö, Camci C, Cakmak EA, Arslan A, Oztuzcu S (2015) The association of the expression of miR-122-5p and its target ADAM10 with human breast cancer. Mol Biol Rep 42: 497-505. doi: 10.1007/s11033-014-3793-2.
36. Saleh AA, Soliman SE, Habib MSE, Gohar SF, Abo-Zeid GS (2019) Potential value of circulatory microRNA122 gene expression as a prognostic and metastatic prediction marker for breast cancer. Mol Biol Rep 46:2809-2818. doi: 10.1007/s11033-019-04727-5

Tables

Table 1. TaqMan mRNA Assay ID numbers

Assay ID	Gene	RefSeq	Exon Boundry	Amplicon ength (bp)
GENEXs300	TMPRSS6	NM_001289001.2	11-12	87
		NM_001289000.2	11-12	87
		NM_153609.4	11-12	87
		NM_001374504.1	11-12	87
GENEXs300	HFE2	NM_213653.4	4-4	89
		NM_001379352.1	4-4	89
		NM_145277.5	3-3	89
		NM_001316767.2	4-4	89
		NM_202004.4	3-3	89
		NM_213652.4	2-2	89
GENEXs300	HAMP	NM_021175.4	1-3	101
GENEXs300	IRP1	NM_001278352.2	20-21	75
		NM_001362840.2	20-21	75
		NM_002197.3	19-20	75
GENEXs300	FPN1	NM_014585.6	5-6	76
GENEXs300	TFRC	NM_003234.4	9-10	82
		NM_001128148.3	9-10	82
		NM_001313965.2	8-9	82
		NM_001313966.2	7-8	82
GENEXs300-C	ACTB	NM_001101.5	4-5	114

Table 2. TaqMan miRNA Assay ID numbers

Gene ID	Accession No	Assay Name and ID No	Amplicon length (bp)
hsa-miR-122-5p	MIMAT0000421	miRNA qPCR SL Assay for hsa-miR-122-5p, MIREX-H122	62-66
hsa-RNU6-6P	Entrez Gene ID: 26826	miRNA qPCR U6 Control Assay for Human RNU6-6P, MIREX-U6	89

Table 3. Expression values and fold changes of genes in tumor and normal tissue samples

Gene	N	Group	ΔCtb (Mean ±SE)	$2^{-\Delta CT}$ (Mean ±SE)	ΔΔCtb (Mean ±SE)	Fold change	P-value
FPN1	75	N	5,2523± 0,1092	0,0331± 0,0028	0,6733± 0,2027	0,627	0,836
		T	5,9256± 0,1992	0,0319± 0,0051			
HAMP	75	N	8,8239± 0,2385	0,0055± 0,0011	0,0488± 0,1928	1,034	0,911
		T	8,8573± 0,2276	0,0057± 0,0012			
HFE2	75	N	6,5880± 0,2838	0,0312± 0,0050	0,9129± 0,2102	0,531	0,368
		T	7,5009± 0,2698	0,0228± 0,0080			
IRP1	75	N	7,7353± 0,1104	0,0055± 0,0004	0,7507± 0,1039	0,594	0,0001
		T	8,4861± 0,1065	0,0034± 0,0003			
TFRC	75	N	7,6217± 0,1061	0,0062± 0,0005	0,6658± 0,1381	1,586	0,0001
		T	6,8808± 0,1448	0,0115± 0,0012			
TMPRSS6	75	N	9,3419± 0,3254	0,0133± 0,0029	0,9353± 0,3720	1,912	0,651
		T	8,4066± 0,3308	0,0190± 0,0121			
miR-122	75	N	2,5377± 1,3422	0,0008± 0,0001	0,0607± 0,7548	0,32	0,025
		T	2,7238± 1,4169	0,0003± 0,0002			

SE: Standart error