CANCER GENOMICS & PROTEOMICS 19: 673-682 (2022)
doi:10.21873/cgp.20350

# Inverse Correlation of KISS1 and KISS1R Expression in Triple-negative Breast Carcinomas from African American Women

MUSTAFA QASIM[1], LUISEL J. RICKS-SANTI[2], TAMMEY J. NAAB[3], FAREED RAJACK[3], DESTA BEYENE[4], MUNEER ABBAS[1], OLAKUNLE O. KASSIM[1], ROBERT L. COPELAND[4,5] and YASMINE KANAAN[1,4]

[1]*Department of Microbiology, Howard University College of Medicine, Washington, DC, U.S.A.;*
[2]*Department of Pharmacotherapy and Translational Research, College of Medicine, University of Florida, Gainesville, FL, U.S.A.;*
[3]*Department of Pathology, Howard University Hospital, Washington, DC, U.S.A.;*
[4]*Howard University Cancer Center, Washington, DC, U.S.A.;*
[5]*Department of Pharmacology, Howard University College of Medicine, Washington, DC, U.S.A.*

**Abstract.** *Background/Aim: The kisspeptin 1 (KISS1) gene encodes a precursor polypeptide which after proteolysis forms the kisspeptin-10 (KISS1) protein. KISS1, retains maximum physiological activity when it binds to its receptor (KISS1R), allowing KISS1 to effectively function as a suppressor of metastasis in melanomas and other types of cancer. The goal of this study was to evaluate the expression of KISS1 and KISS1R in breast carcinomas from African American (AA) women and correlate their association with clinicopathological features, including breast cancer subtypes, and outcomes. Materials and Methods: Tissue microarrays were constructed from formalin-fixed, paraffin-embedded surgical blocks from 216 AA patients. KISS1 and KISS1R expression was assessed using immunohistochemistry. Univariate analysis was used to determine the association between the expression of KISS1 and KISS1R, and clinicopathological characteristics. Pearson correlation was also determined between immunohistochemical H-scores, tumor size, and the number of positive lymph nodes. Kaplan–Meier estimates of overall and disease-free survival were plotted, and log-rank tests were performed to compare estimates among groups. Results: KISS1 protein expression was found to be higher in receptor-negative and triple-negative breast cancer (TNBC) compared to other subtypes ($p<0.001$). However, KISS1R expression was higher in non-TNBC tumors compared to other subtypes ($p<0.001$). Higher KISS1R expression was marginally negatively correlated with tumor size ($p=0.077$), and positively correlated with lymph-node positivity ($p=0.056$), and disease-free survival ($p=0.092$). Conclusion: Our study showed a significant inverse correlation between KISS1 and KISS1R in TNBC. This investigation implicates a role for KISS1 and KISS1R in the pathogenesis of TNBCs in AA women.*

*Correspondence to:* Yasmine M. Kanaan, Department of Microbiology, Cancer Center, College of Medicine, Howard University, 520 W Street, NW, Washington, DC, 20059, U.S.A. Tel: +1 2028069540, Fax: +1 2026671686, e-mail: ymkanaan@howard.edu

*Key Words:* KISS1, KISS1R, Triple-negative breast cancer, African American women.

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY-NC-ND) 4.0 international license (https://creativecommons.org/licenses/by-nc-nd/4.0).

A family of proteins known as kisspeptins play a role in regulating pulsatile hormone production of the hypothalamic–pituitary–gonadal axis. The kisspeptin 1 gene is located at 1q32 of the human genome and is composed of four exons which encode for a single precursor polypeptide (145 amino acids). The precursor peptide undergoes different proteolytic processes creating different kisspeptin derivatives (KP-54, KP-14, KP-13, and KP-10) (1). Of these derivatives, kisspeptin-10 (also known as KP-10 or KISS1) retains maximum physiological activity when binding to its receptor, G-protein-coupled receptor 54 (KISS1R, also known as GPR54).

Despite the many reports of the anti-metastatic function of *KISS1* and *KISS1R* signaling pathway in several types of cancer, their functions in breast cancer are controversial. Many researchers reported the anti-metastatic function of KISS1 and KISS1R (1-5), while others reported their oncogenic and metastatic function (6-13). For example, it was shown that *KISS1* mRNA and protein were not expressed in node-positive tumors compared to node-negative tumors (10), supporting its

anti-metastatic role. Furthermore, KISS1 protein levels were found to be higher in primary breast tumors compared to those which had metastasized to the brain (10). Nicolle *et al*. also reported that KISS1 significantly reduced expression in metastatic cancer, with KISS1 activating a cascade that suppressed cell migration from the primary tumor site (14). Regarding KISS1R, no significant difference in its expression was found in either node positive or node-negative breast cancer (10).

*In vitro* experiments in estrogen receptor α (ERα)-positive cells (MCF-7 and T47D) also demonstrated the function of KISS1 as an anti-metastatic protein by inhibiting the expression of the oncogenes WASP family member 3 (*WASF3*), a member of Wiskott–Aldrich family of proteins, due to increased levels of zinc finger E-box binding homeobox 1 and 2 (ZEB1/2). Therefore, a reduced level of KISS1 expression results in up-regulation of WASF3 and ZEB1/2 along with increased activity of metallopeptidase- 9 (MMP9), causing cells to acquire an invasive phenotype (3). Conversely, increased levels of *KISS1* mRNA and protein were found in MDA-MB-231 cells and enhanced cellular metastasis and reduced cell adhesion through epidermal growth factor receptor transactivation were demonstrated, which led to endogenous stimulation of KISS1R expression, and stimulation of MMP9 (11). Remarkably, treatment of a non-tumorigenic epithelial cell line MCF-10A with KISS1 caused cells to acquire a mesenchymal-like phenotype in 3D cell cultures while expressing stable levels of KISS1R. It is possible that under pathological conditions and upon loss of ERα, there is up-regulation of KISS1 or KISS1R which might cause epithelial–mesenchymal transition (10).

Studies have investigated the relationship between ERα signaling and KISS1/KISS1R in breast cancer with opposing conclusions. One study of 59 primary breast cancer tissue samples found that KISS1/KISS1R levels were elevated in ERα-positive tumors compared to ERα-negative breast cancer (8). However, another showed that estradiol signaling, in ERα-expressing MDA-MB-231 cells, down-regulated *KISS1* expression by altering the binding of RNA polymerase II at the *KISS1* promoter (15). Conversely, a study of 124 tumor samples revealed higher KISS1 expression in node-positive than in node-negative tumors, and that elevated KISS1 expression induced invasiveness and reduced cell adhesion in MDA-MB-231 cells (6).

Given the debatable roles of *KISS1* and *KISS1R* in breast cancer, there is still a need to characterize these markers in hormone receptor-positive and -negative tumors. We hypothesize that *KISS1* signaling has an anti-metastatic function in the presence of ERα, which maintains epithelial growth and regulates the transcription of KISS1 and KISS1R. We also hypothesize that in the absence of ERα expression, KISS1/KISS1R may be up-regulated resulting in epithelial–mesenchymal transition and promoting a migratory phenotype (invasiveness). Therefore, the goal of this study was to evaluate and correlate the immunohistochemical expression of KISS1 and KISS1R with clinicopathological features and breast cancer subtypes. Because of the high prevalence of receptor-negative tumors in African American (AA) women, we characterized and assessed KISS1 and KISS1R expression in those with triple-negative breast cancer (TNBC).

## Materials and Methods

*Tissue microarrays and immunohistochemistry* (IHC). This study was reviewed and approved by the Howard University Institutional Review Board (IRB-19-MED-53). The breast cancer cases were diagnosed at Howard University Hospital between 1998 and 2013 with >5 years of follow-up. A series of TMAs were constructed (Pantomics, Inc., Richmond, CA, USA) consisting of 10×16 arrays of 1.0-mm tissue cores from well-preserved, morphologically representative tumors in archived formalin-fixed, paraffin-embedded surgical blocks from 216 AA patients with primary intraductal carcinoma. A precision tissue arrayer was used for punching the marked foci on the blocks, which correlated with the optimal tumor on the hematoxylin and eosin-stained sections. The device also had a micrometer-precise coordinate system for tissue assembly on a multi-tissue block. Two separate tissue cores of intraductal carcinoma represented each surgical case in the TMA series. Each separate tissue core was assigned a unique TMA location number, which was subsequently linked to an Institutional Review Board-approved database containing demographic and clinical data. Using a microtome, 5-μm sections were cut from the TMA blocks and mounted onto Superfrost Plus microscope slides.

KISS1 and KISS1R expression were assessed with a rabbit anti-human KISS1 antibody at a 1:200 dilution, and rabbit anti-human GPR54 antibody at a 1:100 dilution (US Biological Life Sciences, Salem, MA, USA). The sections were evaluated for the intensity of reactivity (scored 0-3) and the percentage of reactive cells, cytoplasmic expression for KISS1 and membranous for KISS1R. H-Scores were determined based on the product of the intensity of reactivity and the percentage of cells showing immunochemical expression. Cases were categorized as having negative/weak (score <150 for KISS1 and <100 for KISS1R) expression or moderate/strong (score >10) expression. The final H-score was the average of the duplicate cores.

Breast subtypes were defined using immunohistochemical expression of ER, progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67 index (as a percentage). Luminal A subtype was characterized by strong expression of ER or PR (H-score ≥200) and HER2 negativity. Luminal B subtype was characterized by weaker expression of ER or PR (H-score <200) and HER2 positivity, Ki-67 >14%, or by positive expression of ER, PR and HER2. The HER2-overexpressing subtype was hormone receptor-negative with only HER2 positivity. The triple-negative subtype lacked expression of ER, PR, and HER2.

*Statistical analysis*. Statistical analyses were performed using the SPSS 28 statistical program (IBM, Armonk, NY, USA). IHC results were analyzed as continuous variables (H-scores) and categorical/bivariate variables (negative/weak and positive/moderate/strong) as described in the IHC section. Univariate analysis was utilized to determine the association between IHC markers and clinicopathological variables such as: ER, PR, HER2, subtype, modified Scarff–Bloom–Richardson

Table I. *Clinicopathological data of the study population.*

| Parameters | Category | Frequency, n (%) |
|---|---|---|
| Recurrence | No | 96 (44.4) |
| | Yes | 54 (25.0) |
| | Total | 150 (69.4) |
| | Missing | 66 (30.6) |
| Lymph-node positivity | No | 83 (38.4) |
| | Yes | 85 (39.4) |
| | Total | 168 (77.8) |
| | Missing data | 48 (22.2) |
| Pathological stage | 0 | 16 (7.4) |
| | 1 | 19 (8.8) |
| | 2 | 30 (13.9) |
| | 3 | 16 (7.4) |
| | 4 | 3 (1.4) |
| | Total | 84 (38.9) |
| | Missing data | 132 (61.1) |
| Breast cancer subtype | Luminal A | 45 (20.8) |
| | Luminal B | 1 (0.5) |
| | HER-2 Overexpression | 12 (5.6) |
| | TNBC | 143 (66.2) |
| | Total | 201 (93.1) |
| | Missing | 15 (6.9) |
| | Non-TNBC | 58 (26.9) |
| | TNBC | 143 (66.2) |
| | Total | 201 (93.1) |
| | Missing data | 15 (6.9) |
| ER status | Negative | 157 (72.7) |
| | Positive | 47 (21.8) |
| | Total | 204 (94.4) |
| | Missing data | 12 (5.6) |
| PR status | Negative | 165 (76.4) |
| | Positive | 38 (17.6) |
| | Total | 203 (94) |
| | Missing data | 12 (6) |
| HER2 status | HER2 equivocal | 189 (87.5) |
| | HER2 overexpression | 13 (6) |
| | Total | 202 (93.5) |
| | Missing data | 14 (6.5) |

ER: Estrogen receptor; HER2: human epidermal growth factor receptor 2; PR: progesterone receptor. $HER2^+$: $ER^-$, PR–, $HER2^+$; luminal A: $ER^+$ or $PR^+$, $HER2^-$; luminal B: $ER^+$ or $PR^+$, $HER2^+$; TNBC: triple-negative breast cancer, $ER^-$, $PR^-$, $HER2^-$; Equivocal: HER2 status was not clear; n: number of cases.

grading (16), TNM staging (17), and tumor size. Chi-square analyses were also performed to determine the association between KISS1 and KISS1R IHC expression with breast cancer clinicopathological factors. Analysis of variance (ANOVA) was used to determine the association between KISS1 and KISS1R H-scores and categorical variables. Bivariate correlations using Pearson's correlation were finally performed to determine the correlation between KISS1 and KISS1R H-scores and continuous variables.

Values of $p<0.05$ were considered statistically significant and values of $p\geq0.1$ marginally statistically significant. Kaplan–Meier estimates of overall survival (defined as the length of time from the date of diagnosis until the last day of contact with the patient or death) and disease-free survival (defined as the length of time from the date of diagnosis until the day of breast cancer recurrence) were plotted, and a log-rank test performed to compare estimates among groups. The study covered the period from 1998 to 2013 with >5 years of follow-up.

## Results

*Clinicopathological data of the patients with breast cancer.* Clinical and pathological characteristics of the study population are summarized in Table I. The mean age of the patients at diagnosis ranged from 23 to 97 years. The mean tumor size and median number of positive regional lymph nodes were 35.43 mm, and 11.21; respectively.

Fifty-four (25%) and 96 (44.4%) patients were with and without recurrence of breast cancer, respectively; 83 (38.4%) had lymph node-negative disease while 85 (39.4%) had lymph node-positive disease.

Clinical data were available for 201 patients out of the 216 samples tested in the TMAs. Among the 201 patients, 45 (20.8%) had luminal A, one (0.5%) luminal B, and 12 (5.6%) had HER2-overexpressing breast cancer. These three subtypes were grouped into the non-TNBC category (n=58; 26.9%) and compared to the 143 (66.2%) patients with TNBC.

*KISS1/KISS1R expression in different stages of human breast cancer.* IHC was used to investigate the cytoplasmic and membranous expression levels of KISS1 and KISS1R using the TMAs (Figure 1). Table II presents the descriptive statistical analysis of the continuous variables: KISS1 and KISS1R H-scores, age, tumor size, positive lymph nodes and excised regional lymph nodes. The final scoring of KISS1 and KISS1R protein expression was correlated with the clinicopathological data of the study population. ANOVA showed an inverse relationship between KISS1 and KISS1R expression; increased KISS1 and low KISS1R expression were found in TNBCs while low KISS1 and increased KISS1R expression were seen in non-TNBCs and luminal breast tumors. A high KISS1 H-score was significantly associated with ER-negative ($p<0.001$), PR-negative ($p<0.001$), HER2-negative ($p<0.001$), and TNBC ($p<0.001$) phenotypes (Table III; Figure 2). On the contrary, high membranous expression of KISS1R was significantly associated with ER-positive ($p<0.001$), PR-positive ($p=0.003$), HER2-positive ($p<0.001$) and non-TNBC ($p<0.01$) phenotypes (Table III; Figure 2).

Pearson correlation also found an inverse relationship between KISS1 and KISS1R H-scores ($p<0.001$) (Figure 3).

Pearson's correlation coefficient (two-tailed test) was used to determine whether there was a significant relationship albeit weak correlation between the KISS1/KISS1R H-scores and the continuous variables; scatter plots were used to represent the relationship between the numeric variables. Pearson correlation analysis (Figure 4) showed that high KISS1R expression was marginally associated with smaller tumor size

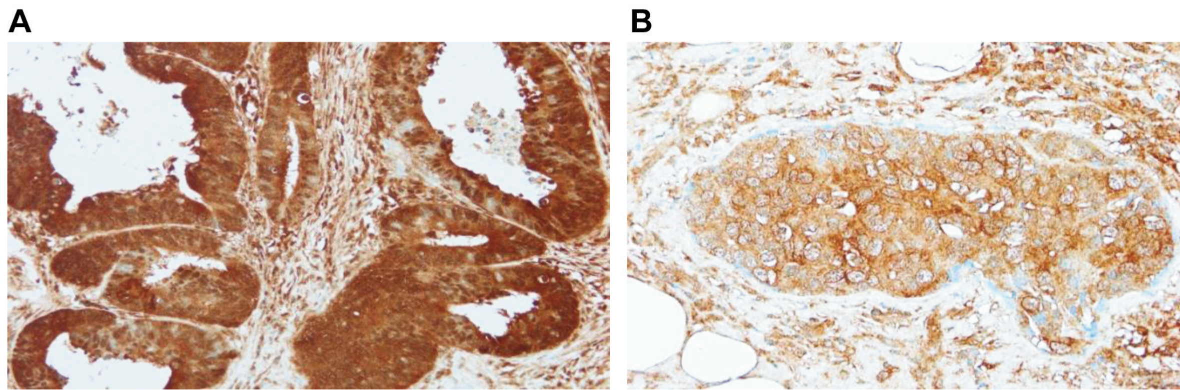


Figure 1. *Immunohistochemical expression of KISS1 and KISS1R in human breast cancer tissues. A: Strong cytoplasmic immunostaining of KISS1 expression (magnification, ×200). B: Strong membranous immunostaining of KISS1R expression (magnification, ×400).*

Table II. *Descriptive statistical analysis of the continuous variables.*

| Variable | N | Minimum | Maximum | Mean | SD |
|---|---|---|---|---|---|
| KISS1 H-score | 172 | 0 | 300 | 146.09 | 103.024 |
| KISS1R H-score | 137 | 0 | 300 | 92.12 | 87.954 |
| Age, years | 200 | 23 | 97 | 57.20 | 14.213 |
| Tumor size, mm | 174 | 3 | 150 | 35.43 | 27.667 |
| Lymph node positivity | 168 | 0 | 97 | 3.70 | 9.878 |
| Excised regional lymph nodes | 189 | 0 | 98 | 11.21 | 13.752 |

N: Number of patients; SD: standard deviation.

(Pearson correlation=–0.169, $p$=0.077), number of positive nodes (Pearson correlation=0.179, $p$=0.056), and better disease-free survival (Pearson correlation=0.216, $p$=0.092).

## Discussion

Literature on breast cancer, the role and the expression of KISS1 and its receptor KISS1R is contradictory. Numerous studies have demonstrated that *KISS1* served as a promising biomarker relative to the diagnosis, identification of therapeutic targets and prognosis in various carcinomas, while other studies have systematically summarized its subjective factors and concluded the functions of KISS-1/KISS1R signaling in physiology homeostasis and cancer biology [reviewed in (18)]. Reports on KISS1 and KISS1R expression have documented the anti-metastatic role of KISS1 in different tumors such as melanoma (19-21), pancreatic (22), brain (23) and esophageal (24) cancer, and in HeLa cells (25).

KISS1 has been shown to be up-regulated in aggressive breast cancers, having adverse outcomes including metastases and death (26). KISS1 is a peptide and a ligand requiring binding to KISS1R to activate the KISS1/KISS1R signaling pathway which suppresses tumor growth, invasion, and metastasis. The intracellular signaling of *KISS1R* has been determined both in human and mouse cell lines in different studies (1-3, 12, 34, 27-30). The receptor is a G-protein-coupled receptor composed of seven transmembrane domains; upon activation, it activates phospholipase C, which mediates the conversion of phosphatidylinositol *bis*phosphate ($PIP_2$) to inositol 1,4,5-triphosphate ($IP_3$) and diacylglycerol. $IP_3$ stimulates the intracellular reserve of calcium ions ($Ca^{2+}$) to be released from the smooth endoplasmic reticulum to the cytoplasm. On the other hand, diacylglycerol along with a high intracellular $Ca^{2+}$ level causes activation of protein kinase C, which in-turn activates other mitogen-activated protein kinases, including extracellular signal-regulated kinases 1 and 2, and P38, depending on the type of cell (31). This is in part the basis for the control of cellular proliferation and migration. Activation of different mitogen-activated protein kinases may contribute to the antiproliferative and anti-metastatic effects of KISS1. However, the expression and signaling cascade of *KISS1*/*KISS1R* cannot be generalized to all types of cells. Therefore, the goal of our study was to evaluate the expression of KISS1 and KISS1R in breast carcinomas from AA women and to determine their association with clinicopathological outcomes including breast cancer subtypes.

Table III. *Analysis of variance of mean cytoplasmic KISS1 and membranous KISS1R immunohistochemical scoring (H-scores), and their association with the breast cancer subtypes of the study population.*

| | | KISS1 (N=162) | | | | KISS1R Expression (N=125) | | | |
|---|---|---|---|---|---|---|---|---|---|
| Factor | Subgroup | Mean H-score | SEM | F | *p*-Value | Mean H-score | SEM | F | *p*-Value |
| ER | Negative | 177.95 | 8.57 | 42.12 | <0.001 | 72.36 | 8.57 | 24.53 | <0.001 |
| | Positive | 71.10 | 12.30 | | | 173.61 | 12.32 | | |
| PR | Negative | 170.97 | 8.57 | 28.38 | <0.001 | 78.35 | 8.57 | 9.50 | 0.003 |
| | Positive | 72.88 | 12.32 | | | 157.50 | 12.32 | | |
| HER2 | Negative | 161.62 | 8.17 | 17.42 | <0.001 | 76.64 | 8.17 | 18.09 | <0.001 |
| | Positive | 40.00 | 10.87 | | | 209.29 | 10.87 | | |
| Subtype | Luminal A | 73.72 | 12.79 | 27.32 | <0.001 | 169.69 | 12.790 | 18.24 | <0.001 |
| | Luminal B | 40.00 | 25 | | | 210.00 | | | |
| | HER2-overexpression | 40.00 | 11.91 | | | 209.17 | 11.91 | | |
| | TNBC | 192.50 | 8.00 | | | 60.98 | 8.00 | | |
| TNBC status | Non-TNBC | 65.78 | 10.26 | 80.74 | <0.001 | 181.74 | 10.26 | 53.45 | <0.001 |
| | TNBC | 192.50 | 8.30 | | | 60.98 | 8.30 | | |

ER: Estrogen receptor; HER2: human epidermal growth factor receptor 2; PR: progesterone receptor; TNBC: triple-negative breast cancer; $HER2^+$: $ER^-$, $PR^-$ and HER2+; luminal A: $ER^+$ or $PR^+$ and $HER2^-$; luminal B: $ER^+$ or $PR^+$ and $HER2^+$; TNBC: $ER^-$, $PR^-$ and $HER2^-$; N: number of cases; SEM: standard error of the mean.

Our study shows a significantly strong inverse correlation between KISS1 and reduced KISS1R. Our IHC data showed an elevated level of KISS1 expression as the TNM stage breast cancer increased, with the highest level being expressed in TNBCs. This finding is similar to what had been previously reported by Martin *et al*. (6), “KISS1 increased with tumor grade and increased TNM status in breast cancer”. Whereas another study of 59 primary breast cancer tissue samples indicated that both KISS1 and KISS1R levels were elevated in ER-positive tumors rather than in ER-negative breast cancer (8). On the other hand, our Pearson correlation analysis revealed an inverse relationship between KISS1 and KISS1R expression. Our findings suggest that there is a negative-feedback mechanism involving the expression of KISS1 and the expression of KISS1R. ANOVA analysis showed higher expression of KISS1R in ER-positive tumors compared to ER-negative tumors and this might explain why the expression of KISS1 is higher in ER-negative tumors to compensate for the low expression level of the receptor (KISS1R). Several studies have reported that the ER status of breast epithelium critically regulates the ability of KISS1R signaling to stimulate the invasiveness of the disease. The ‘brake’ keeping KISS1/KISS1R signaling in check is lost in ER-negative cancer (32, 33); and as a result of the reduced expression of either or both KISS1 and KISS1R; therefore, increased signaling through KISS1R might lead to the induction of the epithelial–mesenchymal transition (34, 35).

Furthermore, we found a positive correlation between KISS1R expression and lymph node-positivity, and a negative correlation with KISS1 expression. As a result, the KISS1R level may be high in lymph-node positive tumors due to KISS1R transactivation by epidermal growth factor receptor, mediated by β-arrestin 1/2 (10), and subsequent increased expression of MMP9 (11). Moreover, KISS1R transactivation may cause suppression of KISS1 expression based on the results mentioned above. Therefore, KISS1R might serve as a biomarker for breast cancer progression and metastasis.

In contrast, an IHC and quantitative reverse transcription-polymerase chain reaction study of 124 tumor samples revealed higher KISS1 expression in node-positive than in node-negative tumors, and elevated KISS1 expression induced invasiveness and reduced cell adhesion in MDA-MB-231 cells (6). However, the KISS1 signaling pathway has an anti-metastatic role in the presence of estradiol signaling, which maintains epithelial growth and regulates transcription of KISS1 and KISS1R, while in the absence of ER expression, transcription of KISS1/KISS1R is up-regulated, causing progression to epithelial–mesenchymal transition and promoting a migratory phenotype (invasiveness) (10, 26).

Tumor cells exhibit the Warburg effect, increasing their glucose uptake, and prefer glycolysis rather than oxidative phosphorylation for ATP (36). Previously, we demonstrated that TNBC tumors have metabolic signatures that distinguish them from ER-positive tumors and this contributes to their distinct clinical phenotypes observed. TNBCs were shown to have increased glycolytic intermediates and lactate production compared to ER-positive tumors. The elevation of nearly all glycolytic intermediates in TNBC *versus* ER-positive tumors suggests an increase in Warburg metabolism in TNBCs (37). Notably, KISS1 takes part in reversing the Warburg effect in tumor cells through the SMAD signaling pathway (38, 39). KISS1/KISS1R signaling only occurs with the binding of KISS1 to KISS1R and is blocked when there

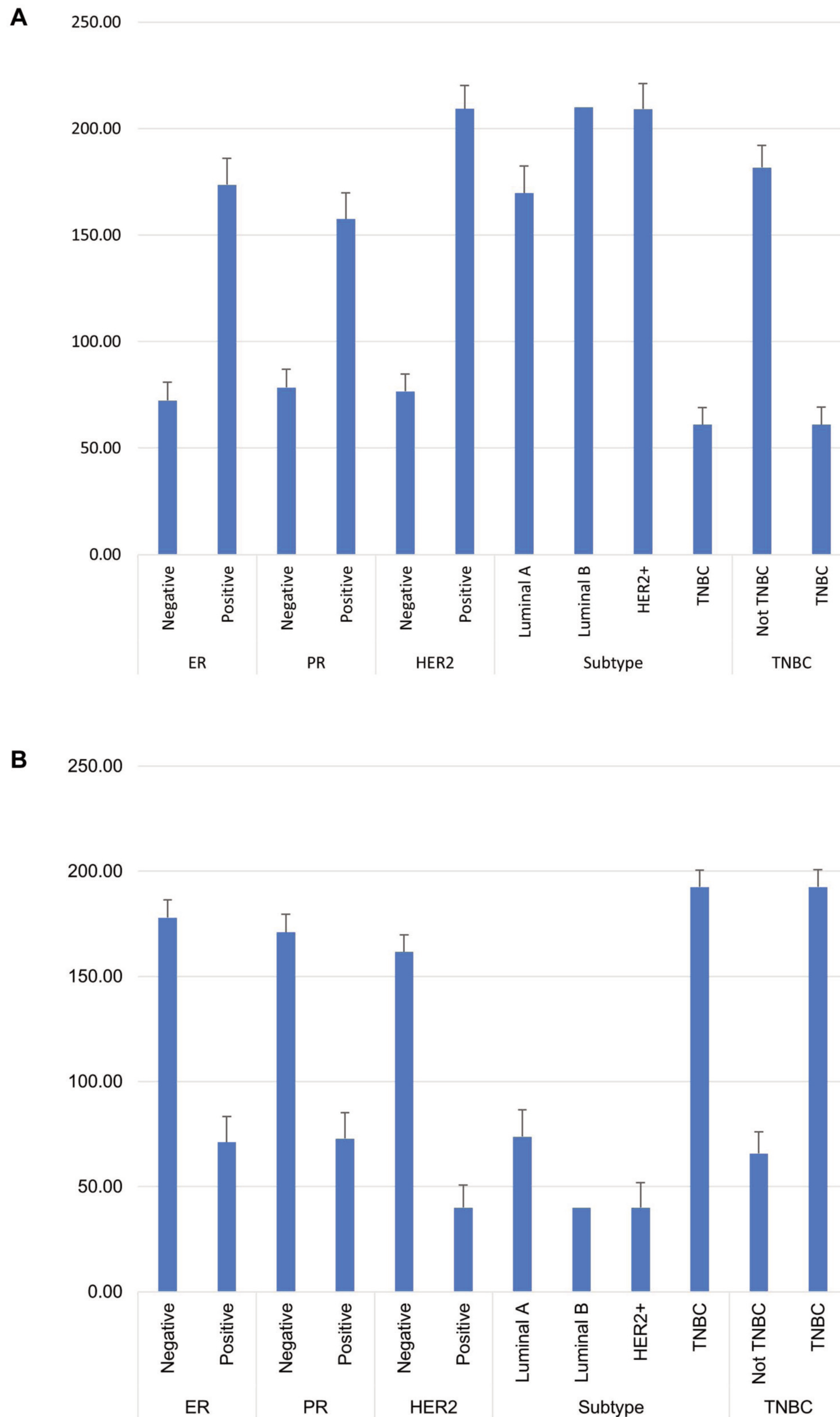


Figure 2. *Analysis of variance of mean membranous KISS1R (A), and cytoplasmic KISS1 (B) immunohistochemical expression (H-scores) by receptor status ER: Estrogen receptor; HER2: human epidermal growth factor receptor 2; PR: progesterone receptor; TNBC: triple-negative breast cancer; HER2$^+$: ER$^–$, PR$^–$ and HER2$^+$; luminal A: ER$^+$ or PR$^+$ and HER2$^–$; luminal B: ER$^+$ or PR$^+$ and HER2$^+$; TNBC: ER$^–$, PR$^–$ and HER2$^–$.*

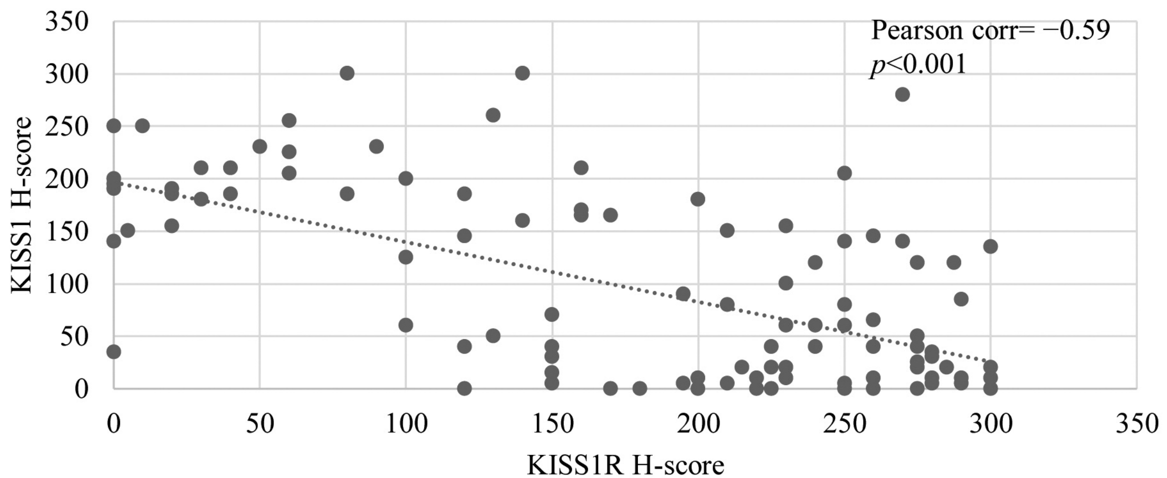


Figure 3. *Correlation between immunohistochemical cytoplasmic KISS1 and membranous KISS1R expression (H-score) in breast cancer tissues.*

is insufficient KISS1R to bind the excessive amount of KISS1 as is observed in TNBCs in AA women.

We found a marginal association of high KISS1R expression with smaller tumor size, number of positive nodes, and better disease-free survival. This is inconsistent with a study reported by Savvidis and colleagues (40). When they studied the relationship between KISS1/KISS1R expression and tumor progression in differentiated thyroid cancer, they found significantly high KISS1 expression in tumors with extrathyroidal invasion and advanced stages, statistically significantly low KISS1R expression, and moderate negative correlation with tumor size. Another study reported high KISS1 expression in hepatocellular carcinoma and its statistically significant influence on diminished disease-free and overall survival (41). Therefore, the function and signaling of *KISS1* and *KISS1R* in breast cancer remain controversial and require further investigation.

*Study limitations*. The availability of long-term follow-up information was limited in some cases and may have compromised the analysis. Therefore, more investigation of immunohistochemical findings in primary breast cancers and metastatic sites other than lymph-nodes may yield helpful information about the role of *KISS1/KISS1R* expression as biomarkers in TNBC in AA women.

## Conclusion

Our study showed a striking significant inverse correlation between KISS1 and KISS1R expression. Increased expression of KISS1 is required to prevent further tumor invasiveness and formation of local or distant metastases, and reduced expression of KISS1R seems to attenuate signaling of the *KISS1/KISS1R* system and leads to tumor growth. Therefore, we postulate that in TNBCs in AA women, *KISS1R* may be deleted, mutated, defective, or expression is saturated, leading to loss of signaling in the *KISS1/KISS1R* pathway with consequent increased capacity for tumor invasion and metastatic spread. Tumorigenesis has been reported to differ in minority populations, and in TNBCs in AA women. Our study seems to indicate that anti-metastatic and tumor-suppressant effects of the *KISS1/KISS1R* pathway are lost in AA women with TNBC, allowing aggressive breast cancer to develop.

The loss of function of the KISS1/KISS1R signaling pathway is associated with adverse prognosis in the specific population of AA women with TNBC. If this signaling pathway is shutdown/nonfunctional in AA women with breast cancer, then therapy is needed that up-regulates signaling, *i.e.* using an agonist, instead of an antagonist (down-regulating abnormal signaling) as has been shown in other studies in White females (42, 43).

Therefore, this initial investigation implicates a role of *KISS1/KISS1R* in the pathogenesis of TNBC in AA women which needs to be verified in larger studies including comparison with different ethnic populations.

## Conflicts of Interest

The Authors have no personal or financial conflicts to report.

## Authors' Contributions

Mustafa Qasim: Contributions to study design, data acquisition, analysis, and interpretation of data. Luisel J. Ricks-Santi: Substantial contributions to statistical data analysis, interpretation of data, revised and finalized the article. Tammey J. Naab: IHC and scoring the protein expression (H-

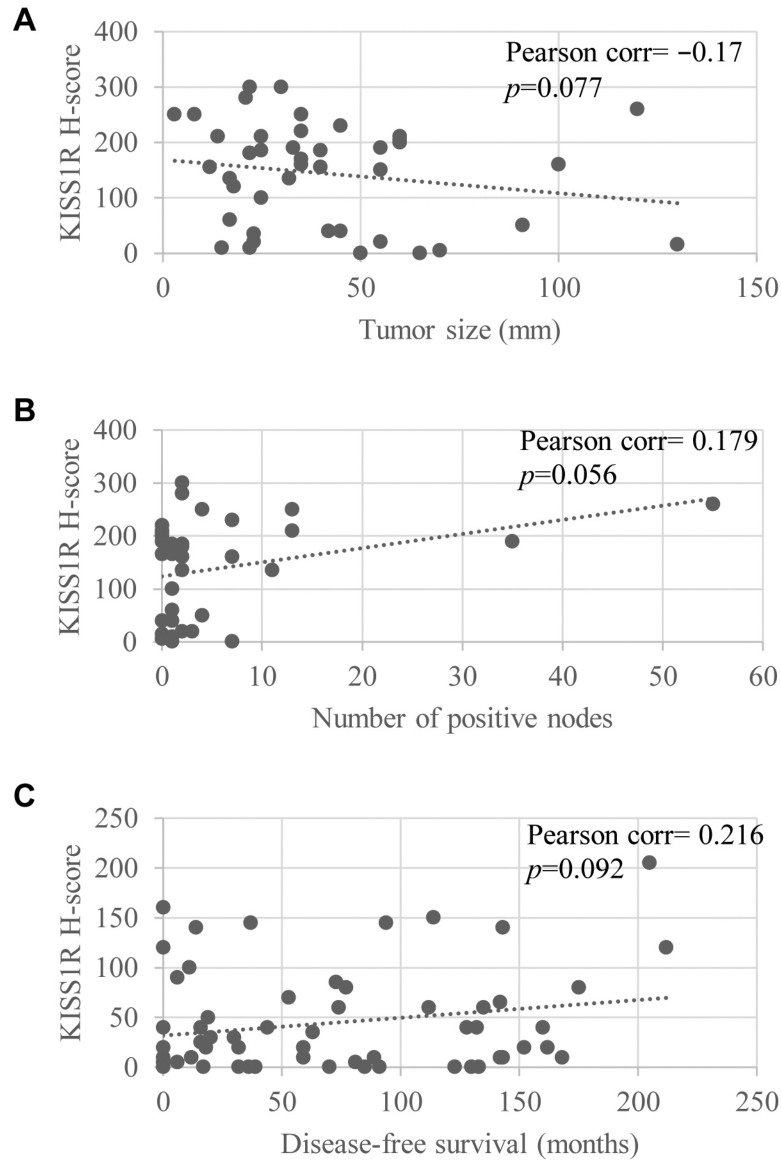


Figure 4. *Correlation between the immunohistochemical membranous KISS1R expression (H-Score) and breast tumor size (A), number of positive lymph nodes (B) and disease-free survival (C). Total number of patients, 110.*

scoring), and data analysis. Fareed Rajack: IHC and scoring the protein expression (H-scoring). Desta Beyene: Data acquisition and interpretation. Muneer Abbas: Revised the article critically for important intellectual content. Olakunle O. Kassim: Revised the article critically for important intellectual content. Robert L. Copeland: Revised the article critically for important intellectual content. Yasmine Kanaan: Substantial contributions to conception, design, data acquisition, analysis, and interpretation of data, drafted and finalized the article.

## Acknowledgements

This work was supported by Charles and Mary Latham Fund (Grant 0009699).

## References

1 Song GQ and Zhao Y: Kisspeptin-10 inhibits the migration of breast cancer cells by regulating epithelial-mesenchymal transition. Oncol Rep *33(2)*: 669-674, 2015. PMID: 25420482. DOI: 10.3892/or.2014.3619

2 Tan K, Cho SG, Luo W, Yi T, Wu X, Siwko S, Liu M and Yuan W: KiSS1-induced GPR54 signaling inhibits breast cancer cell migration and epithelial-mesenchymal transition via protein kinase D1. Curr Mol Med *14(5)*: 652-662, 2014. PMID: 24894166. DOI: 10.2174/1566524014666140603115314

3 Teng Y, Mei Y, Hawthorn L and Cowell JK: WASF3 regulates miR-200 inactivation by ZEB1 through suppression of KISS1 leading to

increased invasiveness in breast cancer cells. Oncogene *33(2)*: 203-211, 2014. PMID: 23318438. DOI: 10.1038/onc.2012.565

4 Song GQ and Zhao Y: Kisspeptin 10 inhibits the Warburg effect in breast cancer through the Smad signaling pathway: both *in vitro* and *in vivo*. Am J Transl Res *8(1)*: 188-195, 2016. PMID: 27069552.

5 Kim TH and Cho SG: Kisspeptin inhibits cancer growth and metastasis *via* activation of EIF2AK2. Mol Med Rep *16(5)*: 7585-7590, 2017. PMID: 28944853. DOI: 10.3892/mmr.2017.7578

6 Martin TA, Watkins G and Jiang WG: KiSS-1 expression in human breast cancer. Clin Exp Metastasis *22(6)*: 503-511, 2005. PMID: 16320113. DOI: 10.1007/s10585-005-4180-0

7 Zajac M, Law J, Cvetkovic DD, Pampillo M, McColl L, Pape C, Di Guglielmo GM, Postovit LM, Babwah AV and Bhattacharya M: GPR54 (KISS1R) transactivates EGFR to promote breast cancer cell invasiveness. PLoS One *6(6)*: e21599, 2011. PMID: 21738726. DOI: 10.1371/journal.pone.0021599

8 Jarząbek K, Kozłowski L, Milewski R and Wołczyński S: KiSS1/GPR54 and estrogen-related gene expression profiles in primary breast cancer. Oncol Lett *3(4)*: 930-934, 2012. PMID: 22741021. DOI: 10.3892/ol.2012.582

9 Jarzabek K, Koda M, Kozlowski L, Milewski R and Wolczynski S: Immunohistochemical study of KiSS1 and KiSS1R expression in human primary breast cancer: Association with breast cancer receptor status, proliferation markers and clinicopathological features. Histol Histopathol *30(6)*: 715-723, 2015. PMID: 25535062. DOI: 10.14670/HH-30.715

10 Cvetkovic D, Dragan M, Leith SJ, Mir ZM, Leong HS, Pampillo M, Lewis JD, Babwah AV and Bhattacharya M: KISS1R induces invasiveness of estrogen receptor-negative human mammary epithelial and breast cancer cells. Endocrinology *154(6)*: 1999-2014, 2013. PMID: 23525242. DOI: 10.1210/en.2012-2164

11 Goertzen CG, Dragan M, Turley E, Babwah AV and Bhattacharya M: KISS1R signaling promotes invadopodia formation in human breast cancer cell *via* β-arrestin2/ERK. Cell Signal *28(3)*: 165-176, 2016. PMID: 26721186. DOI: 10.1016/j.cellsig.2015.12.010

12 Blake A, Dragan M, Tirona RG, Hardy DB, Brackstone M, Tuck AB, Babwah AV and Bhattacharya M: G protein-coupled KISS1 receptor is overexpressed in triple negative breast cancer and promotes drug resistance. Sci Rep *7*: 46525, 2017. PMID: 28422142. DOI: 10.1038/srep46525

13 Dragan M, Nguyen MU, Guzman S, Goertzen C, Brackstone M, Dhillo WS, Bech PR, Clarke S, Abbara A, Tuck AB, Hess DA, Pine SR, Zong WX, Wondisford FE, Su X, Babwah AV and Bhattacharya M: G protein-coupled kisspeptin receptor induces metabolic reprograming and tumorigenesis in estrogen receptor-negative breast cancer. Cell Death Dis *11(2)*: 106, 2020. PMID: 32034133. DOI: 10.1038/s41419-020-2305-7

14 Nicolle G, Comperat E, Nicolaïew N, Cancel-Tassin G and Cussenot O: Metastin (KISS-1) and metastin-coupled receptor (GPR54) expression in transitional cell carcinoma of the bladder. Ann Oncol *18(3)*: 605-607, 2007. PMID: 17164231. DOI: 10.1093/annonc/mdl421

15 Huijbregts L and de Roux N: KISS1 is down-regulated by 17beta-estradiol in MDA-MB-231 cells through a nonclassical mechanism and loss of ribonucleic acid polymerase II binding at the proximal promoter. Endocrinology *151(8)*: 3764-3772, 2010. PMID: 20534720. DOI: 10.1210/en.2010-0260

16 Elston EW and Ellis IO: Method for grading breast cancer. J Clin Pathol *46(2)*: 189-190, 1993. PMID: 8459046. DOI: 10.1136/jcp.46.2.189-b

17 Giuliano AE, Edge SB and Hortobagyi GN: Eighth edition of the AJCC cancer staging manual: breast cancer. Ann Surg Oncol *25(7)*: 1783-1785, 2018. PMID: 29671136. DOI: 10.1245/s10434-018-6486-6

18 Zhu N, Zhao M, Song Y, Ding L and Ni Y: The KiSS-1/GPR54 system: Essential roles in physiological homeostasis and cancer biology. Genes Dis *9(1)*: 28-40, 2020. PMID: 35005105. DOI: 10.1016/j.gendis.2020.07.008

19 Lee JH and Welch DR: Identification of highly expressed genes in metastasis-suppressed chromosome 6/human malignant melanoma hybrid cells using subtractive hybridization and differential display. Int J Cancer *71(6)*: 1035-1044, 1997. PMID: 9185708. DOI: 10.1002/(sici)1097-0215(19970611)71:6<1035::aid-ijc20>3.0.co;2-b

20 Goldberg SF, Miele ME, Hatta N, Takata M, Paquette-Straub C, Freedman LP and Welch DR: Melanoma metastasis suppression by chromosome 6: evidence for a pathway regulated by CRSP3 and TXNIP. Cancer Res *63(2)*: 432-440, 2003. PMID: 12543799.

21 Shirasaki F, Takata M, Hatta N and Takehara K: Loss of expression of the metastasis suppressor gene KiSS1 during melanoma progression and its association with LOH of chromosome 6q16.3-q23. Cancer Res *61(20)*: 7422-7425, 2001. PMID: 11606374.

22 Masui T, Doi R, Mori T, Toyoda E, Koizumi M, Kami K, Ito D, Peiper SC, Broach JR, Oishi S, Niida A, Fujii N and Imamura M: Metastin and its variant forms suppress migration of pancreatic cancer cells. Biochem Biophys Res Commun *315(1)*: 85-92, 2004. PMID: 15013429. DOI: 10.1016/j.bbrc.2004.01.021

23 Zohrabian VM, Nandu H, Gulati N, Khitrov G, Zhao C, Mohan A, Demattia J, Braun A, Das K, Murali R and Jhanwar-Uniyal M: Gene expression profiling of metastatic brain cancer. Oncol Rep *18(2)*: 321-328, 2007. PMID: 17611651.

24 Ikeguchi M, Yamaguchi K and Kaibara N: Clinical significance of the loss of KiSS-1 and orphan G-protein-coupled receptor (hOT7T175) gene expression in esophageal squamous cell carcinoma. Clin Cancer Res *10(4)*: 1379-1383, 2004. PMID: 14977840. DOI: 10.1158/1078-0432.ccr-1519-02

25 Navenot JM, Wang Z, Chopin M, Fujii N and Peiper SC: Kisspeptin-10-induced signaling of GPR54 negatively regulates chemotactic responses mediated by CXCR4: a potential mechanism for the metastasis suppressor activity of kisspeptins. Cancer Res *65(22)*: 10450-10456, 2005. PMID: 16288036. DOI: 10.1158/0008-5472.CAN-05-1757

26 Cvetković D, Babwah AV and Bhattacharya M: Kisspeptin/KISS1R system in breast cancer. J Cancer *4(8)*: 653-661, 2013. PMID: 24155777. DOI: 10.7150/jca.7626

27 Stallaert W, Brüggemann Y, Sabet O, Baak L, Gattiglio M and Bastiaens PIH: Contact inhibitory Eph signaling suppresses EGF-promoted cell migration by decoupling EGFR activity from vesicular recycling. Sci Signal *11(541)*: eaat0114, 2018. PMID: 30065026. DOI: 10.1126/scisignal.aat0114

28 Tian J, Al-Odaini AA, Wang Y, Korah J, Dai M, Xiao L, Ali S and Lebrun JJ: KiSS1 gene as a novel mediator of TGFβ-mediated cell invasion in triple negative breast cancer. Cell Signal *42*: 1-10, 2018. PMID: 28988968. DOI: 10.1016/j.cellsig.2017.10.002

29 Babwah AV, Pampillo M, Min L, Kaiser UB and Bhattacharya M: Single-cell analyses reveal that KISS1R-expressing cells undergo sustained kisspeptin-induced signaling that is dependent upon an influx of extracellular Ca2+. Endocrinology *153(12)*: 5875-5887, 2012. PMID: 23070548. DOI: 10.1210/en.2012-1747

30 Navenot JM, Fujii N and Peiper SC: KiSS1 metastasis suppressor gene product induces suppression of tyrosine kinase receptor signaling to Akt, tumor necrosis factor family ligand expression, and apoptosis. Mol Pharmacol *75(5)*: 1074-1083, 2009. PMID: 19201817. DOI: 10.1124/mol.108.054270

31 Pinilla L, Aguilar E, Dieguez C, Millar RP and Tena-Sempere M: Kisspeptins and reproduction: physiological roles and regulatory mechanisms. Physiol Rev *92(3)*: 1235-1316, 2012. PMID: 22811428. DOI: 10.1152/physrev.00037.2010

32 Izadi P, Noruzinia M, Karimipoor M, Karbassian MH and Akbari MT: Promoter hypermethylation of estrogen receptor alpha gene is correlated to estrogen receptor negativity in Iranian patients with sporadic breast cancer. Cell J *14(2)*: 102-109, 2012. PMID: 23508069.

33 Shi JF, Li XJ, Si XX, Li AD, Ding HJ, Han X and Sun YJ: ERα positively regulated DNMT1 expression by binding to the gene promoter region in human breast cancer MCF-7 cells. Biochem Biophys Res Commun *427(1)*: 47-53, 2012. PMID: 22975348. DOI: 10.1016/j.bbrc.2012.08.144

34 Guttilla IK, Adams BD and White BA: ERα, microRNAs, and the epithelial-mesenchymal transition in breast cancer. Trends Endocrinol Metab *23(2)*: 73-82, 2012. PMID: 22257677. DOI: 10.1016/j.tem.2011.12.001

35 Brisken C and O'Malley B: Hormone action in the mammary gland. Cold Spring Harb Perspect Biol *2(12)*: a003178, 2010. PMID: 20739412. DOI: 10.1101/cshperspect.a003178

36 Hsu PP and Sabatini DM: Cancer cell metabolism: Warburg and beyond. Cell *134(5)*: 703-707, 2008. PMID: 18775299. DOI: 10.1016/j.cell.2008.08.021

37 Kanaan YM, Sampey BP, Beyene D, Esnakula AK, Naab TJ, Ricks-Santi LJ, Dasi S, Day A, Blackman KW, Frederick W, Copeland RL Sr, Gabrielson E and Dewitty RL Jr: Metabolic profile of triple-negative breast cancer in African-American women reveals potential biomarkers of aggressive disease. Cancer Genomics Proteomics *11(6)*: 279-294, 2014. PMID: 25422359.

38 Song GQ and Zhao Y: Kisspeptin 10 inhibits the Warburg effect in breast cancer through the Smad signaling pathway: both *in vitro* and *in vivo*. Am J Transl Res *8(1)*: 188-195, 2016. PMID: 27069552.

39 Liu W, Beck BH, Vaidya KS, Nash KT, Feeley KP, Ballinger SW, Pounds KM, Denning WL, Diers AR, Landar A, Dhar A, Iwakuma T and Welch DR: Metastasis suppressor KISS1 seems to reverse the Warburg effect by enhancing mitochondrial biogenesis. Cancer Res *74(3)*: 954-963, 2014. PMID: 24351292. DOI: 10.1158/0008-5472.CAN-13-1183

40 Savvidis C, Papaoiconomou E, Petraki C, Msaouel P and Koutsilieris M: The role of KISS1/KISS1R system in tumor growth and invasion of differentiated thyroid cancer. Anticancer Res *35(2)*: 819-826, 2015. PMID: 25667462.

41 Schmid K, Wang X, Haitel A, Sieghart W, Peck-Radosavljevic M, Bodingbauer M, Rasoul-Rockenschaub S and Wrba F: KiSS-1 overexpression as an independent prognostic marker in hepatocellular carcinoma: an immunohistochemical study. Virchows Arch *450(2)*: 143-149, 2007. PMID: 17216189. DOI: 10.1007/s00428-006-0352-9

42 Ciaramella V, Della Corte CM, Ciardiello F and Morgillo F: Kisspeptin and cancer: Molecular interaction, biological functions, and future perspectives. Front Endocrinol (Lausanne) *9*: 115, 2018. PMID: 29662466. DOI: 10.3389/fendo.2018.00115

43 Papaoiconomou E, Lymperi M, Petraki C, Philippou A, Msaouel P, Michalopoulou F, Kafiri G, Vassilakos G, Zografos G and Koutsilieris M: Kiss-1/GPR54 protein expression in breast cancer. Anticancer Res *34(3)*: 1401-1407, 2014. PMID: 24596387


*Received May 23, 2022*
*Revised June 29, 2022*
*Accepted July 21, 2022*