

## *Morphology*

# Immunohistochemical Expression of KISS-1 Protein and KISS-1R in Breast Cancer

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Numerous studies have shown that the *kiss-1* gene countervails the metastatic aptitude of several cancer cell lines and solid-tumor neoplasms. However, there still remains ambiguity regarding its role in breast cancer and literature has arisen asserting that Kiss-1 protein may be linked to an aggressive phenotype and malignant progression. We investigated the localization of KISS-1 and its receptor KISS-1R in breast cancer tissue compared to non-cancerous mammary tissue. Immunohistochemical localization was investigated using rabbit polyclonal antibodies against KISS-1 and KISS-1R. A total of 30 tumor formations and 20 samples from non-tumorous mammary tissue were examined. Our study showed that KISS-1 and KISS-1R proteins were significantly higher in breast cancer compared to normal tissue. These results further support the role of the KISS-1/ KISS-1R system in breast cancer biology and might be useful in developing effective therapeutic strategies aimed at modulating the KISS-1/KISS-1R pathway.

*Key words:* KISS-1, KISS-1R, breast cancer

## Introduction

Breast cancer is a progressive and potentially fatal disease that affects women of all ages. It is the most common type of cancer and the leading cause of cancer-related deaths in women in most developed countries [14]. Its high mortality rate is in fact not due to the primary breast tumor burden but rather its metastatic deposits. The metastatic spread of cancer cells is a complex and sequential process that requires detachment from the primary site, survival in the circulation, attachment to and invasion of distant tissues [7]. Tumor cell invasion and metastasis involves genetic and epigenetic modifications of numerous molecular mediators, leading to alterations in various signaling pathways. Numerous studies have shown that the KISS-1 gene countervails the metastatic aptitude of several cancer cell lines and solid-

tumor neoplasias. However, there still remains ambiguity regarding its role in breast cancer and literature has arisen asserting that KISS-1 expression may be linked to an aggressive phenotype and malignant progression.

The *kiss-1* gene encodes a group of biologically active peptides collectively known as kisspeptins (KPs) which play a major regulatory role in puberty onset and reproductive function [1, 3, 8, 28]. Kisspeptins are the endogenous ligands for the G protein-coupled receptor KISS-1R [12]. Activation of this pathway results in stimulation of the phosphatidylinositol-3-kinase/Akt and mitogen activated protein kinase (MAPK) pathways [19].

In non-malignant tissues, KISS-1 expression has been shown to be particularly abundant in the placenta [22], contributing to trophoblast invasion during pregnancy [2, 10, 13, 26], followed by a widespread expression in several central nervous system regions and moderate-to-weak expression in the testis, pancreas, liver, intestine, kidney, lungs and prostate [16, 22, 25]. This tissue distribution pattern is compatible with mRNA localization of GPR54, which is highly expressed in the placenta and central nervous system, and less prominent in intestine, kidney, lungs, and prostate [16, 22, 25]. KISS-1 and KISS-1R expression has been investigated in a variety of cancers including gestational trophoblastic disease [5], melanoma [16, 31], breast cancer [30, 17], hepatocellular carcinoma [11], pancreatic cancer [23], gastric carcinoma [6], esophageal carcinoma [12], papillary thyroid cancer [27], bladder cancer [29], ovarian cancer [15, 9], prostate cancer [32] and pheochromocytoma [24]. The KISS-1/GPR54 system has also been implicated in the pathophysiology of endometriosis [18]. In most malignancies, the *kiss-1* gene seems to act as an anti-metastatic agent and loss of KISS-1 expression is associated with tumor progression and advanced disease. In breast cancer, however, the role of kisspeptin remains elusive due to limited and conflicting data, and it is possible that increased KISS-1 expression correlates with disease progression and poor patient prognosis in this particular type of cancer.

The present study aimed at determining KISS-1 and KISS-1R protein expression in breast cancer tissues compared to non-malignant mammary tissues.

## Materials and Methods

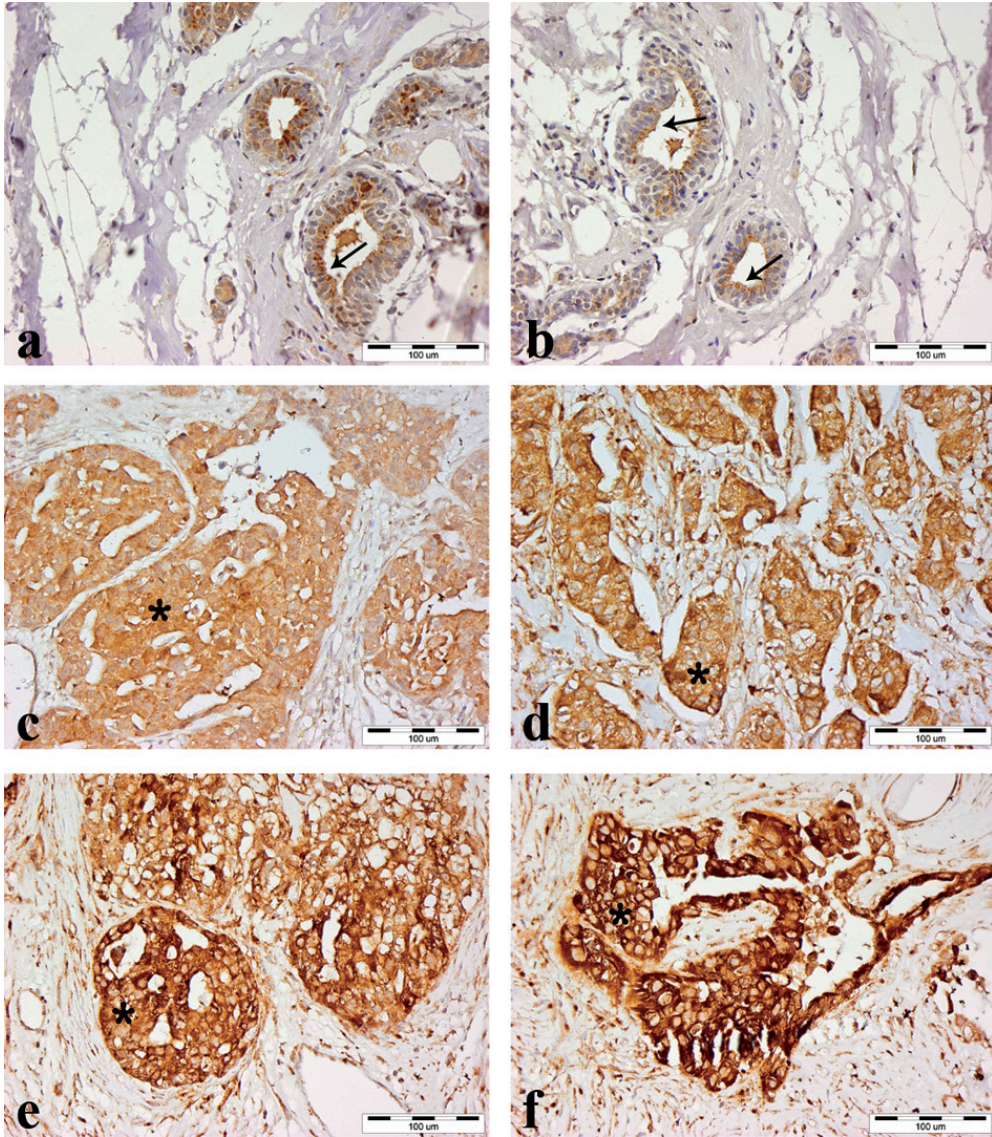
Thirty samples of invasive ductal carcinoma (18 cases of moderately differentiated ductal carcinoma and 12 cases of low-differentiated ductal carcinoma) and 20 samples from non-tumorous mammary tissue, were included in the study.

**Immunohistochemical (IHC) method.** Tissue samples were fixed in 10% buffered formalin, dehydrated and embedded in paraffin. Paraffin sections, 5 µm thick, were stained with haematoxylin and eosin for histopathological evaluation. Antigen retrieval was performed in Citrate Buffer, pH 6.0 (ScyTek Laboratories Inc., USA) at 95°C for 20 min. Endogenous peroxidase activity was blocked with 3% H<sub>2</sub>O<sub>2</sub> for 10 min at room temperature. Subsequently, the sections were washed in TTBS (tris-buffered saline + 0.05% Tween 20) and incubated with primary antibodies against KISS-1 (1:150, rabbit polyclonal, Elabscience Biotechnology Inc., USA) and KISS-1R (1:150, rabbit polyclonal, Elabscience Biotechnology Inc., USA). Biotin-Streptavidin HRP detection system (ScyTek Laboratories Inc., USA) with DAB as chromogen was used.

## Results

The intensity of the reaction was significantly higher in tumours compared to normal tissue. KISS-1 and KISS-1R showed stronger staining in the cytoplasm of tumour cells

with additional membrane reaction for KISS-1R. In low-differentiated invasive ductal carcinomas the reaction was stronger compared to that in moderately differentiated ductal carcinomas (**Fig. 1**).



**Fig. 1.** Immunohistochemical expression of KISS-1 (a, c, e) and KISS-1R (b, d, f) in normal breast epithelium (a, b), invasive moderately differentiated ductal carcinoma (c, d) and invasive low-differentiated ductal carcinoma (e, f). Weak cytoplasmic and more intensive apical reaction for both KISS-1 and KISS-1R in normal breast epithelium was observed (arrows). Stronger staining of the cytoplasm of tumor cells in invasive moderately differentiated ductal carcinomas and low-differentiated ductal carcinoma (asterisk).



## Discussion

The class of proteins known as metastasis suppressors can prevent metastasis without affecting the growth of primary tumour and has recently attracted much attention as it may provide useful mechanistic insight for the development of targeted-therapeutic strategies including drug induced restoration of metastasis suppressor genes and emerged pathways. The *kiss-1* gene was initially identified as a candidate metastasis suppressor in 1996, when it was found that its expression was differentially up-regulated in C8161 melanoma cells that were rendered non-metastatic by microcell-mediated transfer of an intact copy of the human chromosome. However, even though the *kiss-1* gene has been identified as a strong suppressor of metastasis in a variety of cancers, limited and conflicting data have been subjected to the intertwined relationship between KISS-1 system and breast cancer, and its biological role in that particular cancer remains to be elucidated [11, 21, 20]. Reports on the expression of KISS-1 and KISS-1R in different types of tumours are quite controversial. In some studies, KISS-1 expression has been shown to decrease in high-grade tumours, which is associated with increased metastatic potential and unfavourable prognosis. Such relation has been found in gastric adenocarcinoma [11], oesophageal carcinoma [33], ovarian carcinoma [9], etc. Studies on breast cancer, however, have demonstrated the opposite phenomenon. Higher-grade tumours show increased expression of KISS-1 and KISS-1R, which correlates with increased metastatic potential and poor prognosis [35]. Our results show that the expression of KISS-1 and KISS-1R is higher in cancer tissue compared to non-malignant breast tissue. The expression of KISS-1 was also significantly and positively related to the expression of KISS-1R. Our findings are consistent with previous studies conducted by Martin et al. [21] and Marot et al. [20], that have also demonstrated higher expression of KISS-1 in breast cancer compared to normal breast parenchyma. It has been previously shown that ER $\alpha$  plays a key role in controlling KISS-1/KISS-1R signalling. Under physiological conditions, in the presence of ER $\alpha$ , the activation of KISS-1R is associated with growth and remodelling of the gland. Loss of ER $\alpha$  in breast cancer, however, results in increased transcription of KISS-1 and/or KISS-1R. Increased transcription in these cases is not associated with the physiological role of KISS-1 as a metastatic suppressor, but rather, it stimulates invasiveness by EGFR transactivation by KISS-1R. This ligand-independent activation of EGFR further stimulates cellular invasiveness in breast cancer. Furthermore, the transactivation of EGFR by KISS-1R is necessary for the secretion and activation of MMP-9 [35], and the effects of active MMP-9 are associated with stimulation of tumour cell invasion and angiogenesis [34]. Increased KISS-1R signalling in breast cancer has also been associated with induction of EMT, which enables them to assume a mesenchymal phenotype, which includes enhanced migratory capacity, invasiveness, elevated resistance to apoptosis, and greatly increased production of ECM components [4].

## Conclusions

In conclusion, present study demonstrated that protein expression of both KISS-1 and KISS-1R was significantly higher in invasive breast cancer compared to normal breast tissue. The lower tumor differentiation was, the higher KISS-1 and KISS-1R expression was observed. These results further support the role of the KISS-1/KISS-1R system in breast cancer biology and might be useful in developing effective therapeutic strategies aimed at modulating the KISS-1/KISS-1R pathway.

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