MINI-REVIEW

Kisspeptins (*KiSS-1*): Essential Players in Suppressing Tumor Metastasis

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Abstract

Kisspeptins (KPs) encoded by the *KiSS-1* gene are C-terminally amidated peptide products, including KP-10, KP-13, KP-14 and KP-54, which are endogenous agonists for the G-protein coupled receptor-54 (GPR54). Functional analyses have demonstrated fundamental roles of *KiSS-1* in whole body homeostasis including sexual differentiation of brain, action on sex steroids and metabolic regulation of fertility essential for human puberty and maintenance of adult reproduction. In addition, intensive recent investigations have provided substantial evidence suggesting roles of Kisspeptin signalling via its receptor GPR54 in the suppression of metastasis with a variety of cancers. The present review highlights the latest studies regarding the role of Kisspeptins and the *KiSS-1* gene in tumor progression and also suggests targeting the *KiSS-1*/GPR54 system may represent a novel therapeutic approach for cancers. Further investigations are essential to elucidate the complex pathways regulated by the Kisspeptins and how these pathways might be involved in the suppression of metastasis across a range of cancers.

Keywords: *Kiss*peptins - GPR54 receptor - *KiSS-1* gene - metastasis - cancer

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Introduction

Cancer is a class of disease characterized by out-of-control cell growth. Cancer disease is a leading cause of death and accounted for 7.6 million deaths in 2008 and could rise to 13.1 million deaths by year 2030 (WHO, 2008). The reason behind such high mortality from cancer is due to their extremely invasive behaviour which typically results in metastasis (Khan and Mukhtar, 2010).

Metastasis is an extremely complex process that remains to be a major problem in the management of cancer (Hunter et al., 2008). Metastasis is a multistep process, which includes loss of cell-cell adhesion within neoplastic epithelium, invasion of surrounding tissues, angiogenesis and dissemination of cancer cells through vascular and lymphatic vessels, ranspassing through vascular endothelium into distant sites (Hanahan and Weinberg, 2011; Sakthivel et al., 2012). Each step must be successfully completed to give rise to a metastatic tumor. Now-a-days, cancer research is focusing on the blockade of the metastatic process at its early stage.

Therefore, there is growing interest in identifying metastasis suppressor genes, which may be involved in the anti-metastatic activity. Numerous experimental studies reveals that understanding the expression and mechanism of genes and gene products that inhibits cancer metastasis could be a potential molecular target in preventing cancer deaths (Stafford et al., 2008). There are around 20 different metastasis suppressor genes which brings a valuable mechanistic insight for guiding specific therapeutic strategies for cancer including gene transfer, induction of previously suppressed gene expression, exogenous administration of gene product, drug-induced reactivation of metastasis suppressor genes and signalling pathways.

Among metastasis-suppressor genes, *KiSS-1* is the only one that binds to a G-protein coupled receptor (GPR54 or AXOR12 or hOT7T175) and is believed to act late in the metastatic cascade by preventing growth of the metastatic deposit, as opposed to early metastasis suppressor genes [Non metastasis genes (Nm23, *KAI1*)] that suppress cell detachment and migration from the primary tumor (Bari et al., 2009; Prabhu et al., 2012). *KiSS-1* (formerly known as metastin) occur naturally in human placenta which was named for its role as a suppressor sequence (SS); the letters *"Ki"* were appended to the prefix *"SS"* to form "*KiSS*" in homage to the location of its discovery, Hershey, Pennsylvania, home of the famous "Hershey Chocolate *KiSS*es" (Kotani et al., 2001; Shevde et al., 2003; Iiizumi et al., 2008; Smith and Theodorescu, 2009). It is well known that *KiSS-1* gene involve in tumor biology and metastatic process, therefore, in this review we discuss the potential roles of the *KiSS-1* gene during tumor progression and how it could be used as a potential molecular target for cancer therapy.

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Discovery of Kisspeptins and *KiSS-1*

KiSS-1 gene was discovered in1996 in many mammalian species including rat, mouse and recently identified in fish species. Kisspeptins are C-terminally amidated peptide products encoded by the *KiSS-1* gene which is located in long arm of human chromosome 1q32 having four exons consisting of 5' and 3' exons. In humans, this ligand derives from precursor peptide of 145 amino acids in length, with a putative 19 amino acids signal sequence, two potential dibasic cleavage sites, and one putative site for terminal cleavage and amidation. The precursor generates different peptides by photolytic cleavage; thus KPs (also called metastin), is the major peptide that are found to be unstable and may be proteolytically cleaved into the shorter products such as KP-10, KP-13, KP-14 and KP-54 (Smith et al., 2006; Makri et al., 2008).

In this review, these peptides will be collectively referred as Kisspeptins (KPs). Human *KiSS-1* gene contains a Phe residue the C-terminal amidated amino acid, while rodents *KiSS-1* possess a Tyr residue. The human KP-10 peptide contains Tyr at position one, Trp at position three and Ser at position five. Kisspeptin is a ligand for G-protein coupled receptor (GPR54). All KPs binds to the G-protein coupled receptor (GPCR) and GPR54 exhibits modest sequence identity (34-35%) with the galanin receptors GalR1 and GalR2, but is not activated by galanin. The *KiSS-1* gene belongs to a larger family of RFamide (Arg-Phe-NH2) peptide ligand that can activate the human ortholog (hOT7T175 or AXOR12) of GPR54, also termed as *KiSS-1* gene plays a major role in regulation of hypothalamic pituitary gonadal (HPG) axis via regulating the secretion of gonadotropin releasing hormone (GnRH) in the hypothalamus. The greatest peripheral source of KPs in the human is placenta and *KiSS-1* is located within the syncytiotrophoblast cells (Bilban et al., 2004). Studies on functional analysis have demonstrated the fundamental role of *KiSS-1* in multiple process include i.e. sexual differentiation of brain, regulation of gonadotropin secretion via gonadotropin-releasing hormone (GnRH) neurons, action on sex steroids, metabolic regulation of fertility, essential for initiation of human puberty and also maintenance of adult reproduction (Roa and Tena, 2007; 2008; Oakley et al., 2009; Pineda et al., 2010; Hameed et al., 2011; Tena-Sempere et al., 2012). These divulge of *KiSS-1* gene and KPs regulate a wider range of effects in the body and interestingly its role in the suppression of metastasis in a variety of cancers appears to be the most important site of action (Makri et al., 2008; Li et al., 2012).

KiSS-1 in Cancer Progression

The expression profile and the role of *KiSS-1* gene in cancer progression are largely unknown in most of the cancers (Dhar et al., 2004). GPR54 activation by KPs stimulates phosphatidyl inositol 4,5-bisphosphate hydrolysis, calcium mobilization, arachidonic acid release, and ERK1/2 MAPK phosphorylation and has been shown to inhibit cell motility, invasion, proliferation and metastasis (Babwah et al., 2012). However, the precise mechanism of association in tumor metastasis of KPs and GPR54 is still unclear. *KiSS-1* became the strongest independent prognostic factor among the conventional prognosticators for gastric cancer patients (Dhar et al., 2004). Hata et al. (2007) reported that low level of *KiSS-1* gene expression was associated with more aggressive ovarian cancer cell invasion and also increased in patient's prognosis. Martin, (2005) reported that over expression of *KiSS-1* gene increased the tumor progression during breast cancer. The role of *KiSS-1* gene in tumor progression was correlated with over expression of *KiSS-1* and GPR54 genes which are found to observed at all advance stages of tumor progression during hepatocellular carcinoma (Ikeguchi et al., 2003; Shengbing et al., 2009). The expression of *KiSS-1* was higher in pancreatic cancer results in higher cancer invasion suggesting *KiSS* can be possible inhibitor of pancreatic cancer invasion (Liang et al., 2007). Dhar et al. (2004) first reported that down regulation of *KiSS-1* expression produced frequent tumor invasion and it became a strong prognostic determinant in gastric carcinoma patients. Although *KiSS-1* expression level was lower in gastric cancers when compared with normal gastric mucosa, there was a trend of statistical significance between the groups. The author further reveals that this may be due to frequent metaplastic changes in gastric mucosa adjacent to the tumors and might have affected the *KiSS-1* gene expression in gastric mucosa.

Homozygous deletion, promoter methylation, or mutation of *KiSS-1* gene might be responsible for down regulation of this gene in tumor, responsible for increase in tumor invasion. Similar results were also reported earlier by Sanchez-Carbayo et al. (2003), where low expression of *KiSS-1* gene exhibited frequent vascular invasion observed in urinary bladder carcinoma. Therefore *KiSS-1* gene expression became an important evocator of cancer patient survival in terms of overall and disease-free survival. Stafford et al. (2002) reported that peptide inhibits cellular proliferation through intracellular Ca^{++} release and activation of protein kinase C. An increase in intracellular Ca^{++} was found to inhibit cell proliferation and induce cell differentiation and apoptosis in cancer cells. Therefore evidence claims that *KiSS-1* gene continues to be the strongest independent evocator for cancer patient survival. A probable survival supremacy for *KiSS-1* gene expression could be attributable to its tumor suppressor role through the prevention of cellular invasion, metastasis and tumor recurrence. Also *KiSS-1* has an anti-proliferative effect on cancer cells and could be responsible for decreased tumor growth and increased in cancer patient survival (Dhar et al., 2004). However, more studies are necessary to determine the emergent pathway behind the *KiSS-1* gene against all cancers which will shed a new era of cancer treatment by inhibiting the cancer cell invasion.

KiSS-1, as a Molecular Target for Cancer

KiSS-1 gene was identified as a novel human metastatic suppressor gene after the microcell mediated transfer

of intact copy of whole human chromosome 6 into the metastatic human melanoma cell line C8161 without affecting tumourigenicity (Ohtaki et al., 2001; Lee et al., 1996). *KiSS-1* gene encodes a carboxyterminal amidated peptide with 54 amino-acid residues which has G-protein-coupled receptor (GPR54, AXOR12 and hOT7T175) also named as metastin. The characterization of the major product of the *KiSS-1* gene amino acid peptide 'metastin' by virtue of its therapeutic modality that inhibit metastasis of melanoma cells (Funes et al., 2003; Takeda et al., 2012). *KiSS-1* gene expression are found in many metastatic melanoma cell lines such as A375SM, WM2664, WM239A, MeWo and strong signals in normal melanocyte FM1085 which blocks the ability of cancer cells to colonize secondary sites by maintaining disseminated tumor cells in a dormant state after they have lodged within the secondary site (Li et al., 2009; Thiolloy et al., 2011). *KiSS-1* genes are differential in expression and inversely correlated with metastatic potential of melanoma (Takeda et al., 2012).

Recently, a significant reduction in *KiSS-1* or metastin expression has been reported in tumor with high metastatic potential (Sanchez-Carbayo et al., 2003; Dhar et al., 2004; Ikeguchi et al., 2004; Li et al., 2012). The loss or decreased in the expression of CRSP3/DRIP130, a transcriptional co-activator decreased the expression of *KiSS-1* gene results in high metastatic potential during cancer progression (Goldberg et al., 2003). Interestingly it was also found that, the Thioredoxin (mapped in chromosome 1) is a class of small redox proteins plays a role in redox signaling have associated with *KiSS-1* gene up regulation for the inhibition of metastasis (Goldberg et al., 2003).

This information affords more evidence to correlate the role of *KiSS-1* gene difference in expression during tumor progression. Previous studies reported that the expression of *KiSS-1* gene was significantly increased in tumor as when compared to normal tissues as well as in lymph nodes positive tumors as compared to lymph nodes negative ones (Martin et al., 2005). The expression of *KiSS-1* gene was higher and reduction in its receptor (GPR54) in the highly progressive cancer patients (Martin et al., 2005). In an experimental study, during oesophageal carcinoma, the loss of *KiSS-1* and/or GPR54 is correlated to tumor spread to the lymph nodes that determines the depth of tumor invasion (Ikeguchi et al., 2004). The author further reveals that over-expression of *KiSS-1* and GPR54 genes are found to in all advanced-stages of tumor development. *KiSS-1* and GPR54 expression were studied in thyroid cancer and found that the *KiSS-1* and GPR54 are expressed low in follicular cancers which forms distance metastasis (Ringel et al., 2002). Another hypothesis by Hata et al. (2007) revealed that, the decreased expression level of KP-54 was reported to be associated with more aggressive ovarian cancer progression and worse patient prognosis. This study provides validity that *KiSS-1*/GPR54 can reduce cancer cell invasion. Similarly, a study reported that mRNA expression of *KiSS-1* gene inhibited brain metastases from breast cancer (Stark et al., 2005).

It was reported that decreased expression of *KiSS-1* gene results in increased metastatic potential and aggressive tumor progression during gastric cancer (Dhar et al., 2004). Yu, (2007) have studied the protein expression of non-metastatic genes of *KiSS-1*, *KAI-1*, nm23 and p53 in gastric tumors with and without lymph node and liver metastases. The author reported that *KiSS-1*, *KAI1* and nm23 levels were drastically reduced in the metastatic tumors compared to tumor at primary site.

Shengbing et al. (2009) demonstrated the role of *KiSS-1* gene in tumor invasion as well as in intra-hepatic metastasis and distance metastasis by down regulation of *KiSS-1*. The authors propose that *KiSS-1* gene expression could be a useful prognostic marker in hepatocellular carcinoms (HCC) patients to diagnose the early onset of metastasis and design appropriate therapeutic strategies. Sanchez-Carbayo, (2003) investigated the association between *KiSS-1* expression and the clinic pathologic characteristics of bladder tumors and reported that low *KiSS-1* gene expression was significantly associated with worse overall survival in 69 bladder cancer patients. The expression of *KiSS-1* gene was drastically lower in bladder tumors with vascular invasion compared with normal urothelium. Also the author further reports that all bladder tumors have advanced distant metastases during the loss of *KiSS-1*. Similarly decreased *KiSS-1* gene expression were also confirmed by Takeda et al. (2012) in an separate experiment where they found that, *KiSS-1* gene expression in patients with hematogeneous metastases was significantly lower than in those with lymph node metastases. Interestingly, Kostadima, (2007) demonstrated silencing of *KiSS-1* gene in patients with high-risk of early breast cancer that supports *KiSS-1* gene as metastasis-suppressor gene. The author further reported that *KiSS-1* transcriptional activity in patients with node-negative breast cancer, breast cancer primaries and metastatic deposits as well as in patients with other solid tumors was essential. As molecular alteration occur initially in tumors at localized stages. Therefore restoration of *KiSS-1* gene function holds promise for arresting micrometastatic growth and preventing cancer recurrence. *KiSS-1* gene expression level reportedly to be increased in cancerous tissues as compared to benign tumor (Gao et al., 2007).

Overall, studies about expression profile of *KiSS-1* gene revealed that over-expression of *KiSS-1* and its receptor is correlated with disease progression and *KiSS-1* gene may be a possible metastasis suppressor of cancers such as thyroid cancer, oesophageal carcinoma, urinary bladder cancer, gastric carcinoma, epithelial ovarian cancer and colorectal cancer (Shirasaki et al., 2001; Ringel et al., 2002; Sanchez-Carbayo et al., 2003; Dhar et al., 2004; Ikeguchi et al., 2004; Moya et al., 2013). Therefore, numerous experimental and clinical evidences have accumulated unambiguously; support-*KiSS-1* could be a potential molecular target for the prognostication and treatment of metastasis during cancer progression. Even though, Kisspeptin and GPR-54 system has been positively correlated with increased patient survival in many cancers, but contradictorily, studies reported that *KiSS-1* gene expression were increased in human breast cancer, particularly in patients with aggressive tumor. The author further reports that *KiSS-1* gene promotes metastasis in a human breast cancer cell line in an in vitro study (Martin et al., 2005; Makri et al., 2008). In

Table 1. *KiSS-1*: as an Essential Target in Treatment of Cancer Diseases

Sl No	Types of cancer	Experimental and Clinical evidences	References
1	Skin cancer	Drop in *KiSS-1* gene expression coincides with the rapid drop in 5-year survival of patients with tumors >4mm size.	Shirasaki et al., 2001
2	Thyroid cancer	In well-differentiated thyroid malignancies, papillary carcinomas were more likely to express *KiSS-1* mRNA than follicular carcinoma, (69% vs. 20%, $p<0.05$).	Ringel et al., 2002
3	Bladder cancer	*KiSS-1* mRNA expression was elevated in a cohort of superficial and invasive bladder neoplasms.	Sanchez et al., 2003
4	Esophageal squamous cell carcinoma	*KiSS-1* mRNA expression was evaluated in esophageal squamous cell carcinoma (ESCC). *KiSS-1* expression was lost in 38% of ESCC tumors	Ikeguchi et al., 2004
5	Gastric cancer	Gastric cancers with low *KiSS-1* mRNA had frequent venous invasion, distant metastases, tumor recurrence and a significantly worse overall and disease-free survival has been observed.	Dhar et al., 2004
6	Hepatocellular carcinoma	*KiSS-1* mRNA expression was evaluated in HCC. Ikeguchi et al. (2004) did not find any significant changes in *KiSS-1* gene expression between normal liver and HCC samples, but the lack of correlation with disease cannot be determined because the authors did not include the stage of the clinical specimens examined nor did they report on liver metastases.	Ikeguchi et al., 2004
7	Breast cancer	Programming of *KiSS-1* gene expression in human breast cancer cell lines decreased metastatic spread in mouse xenograft models. Induction of *KiSS-1* gene into a metastatic breast cancer cell line MDA-MB-435 reported >95% suppression of metastases to the lung following orthotopic injection.	Olbrich et al., 2010; Lee et al., 1996

support, it is also reported that in hepatocellular cancer Kisspeptin and GPR-54 system have been correlated with decreased survival (Schmid et al., 2007). Even though, Kisspeptins are not always associated positively with some cancers, currently a large body of evidence suggests that, it does appear to inhibit the cancer cell invasion. Different mechanisms of function include increase in ERK1/2 phosphorylation, decrease in MMP-2 as in the placenta as well as to inhibit the metastatic properties of its chemokine receptor CXCR4 have been hypothesised for this inhibition of metastasis process (Stathatos et al., 2005; Yoshioka et al., 2008). These findings may open the possibility of future clinical application of these proteins, *KiSS-1* for prevention of cancer invasion and metastasis, and thus may improve patient prognosis. Collectively, this information provoked us to evaluate the expression profile of these genes and their prognostic impact on cancer metastasis, which is the most common cause of cancer death worldwide. Nash and Welch, (2006) reported that *KiSS-1* gene expression does not affect initial seeding of metastatic cells to the lung, but prevents tumor cell proliferation after arrival. Although there are some questions which still remain unclear, how *KiSS-1* gene maintains dormancy of disseminated cells at the secondary site? In addition, whether *KiSS-1* gene will show efficacy in treating larger established metastatic lesions?. However, additional research is needed to demarcate the signalling pathways further and to find an association with cancer prognosis. Cancers associated with *KiSS-1* gene and as essential target is presented in (Table 1).

Kisspeptins in Therapeutics

Alternative therapeutic option involves treatment with KPs is feasible because they are secreted. Moreover, systemic administration of KPs could access tumor cells throughout the body. The author reported that metastases to all sites were inhibited by *KiSS-1* gene expression and the utilization of KPs block metastases to all tissues (Beck and Welch, 2010). In therapeutic form the author further reveals that, the safety of *KiSS-1*/KP administration is already proven in humans, Subcutaneous administration of KP54 did not cause any reportable adverse effects but administration of KP54 may regulate endocrine function and the onset of puberty, with activation of the KP receptor triggering the release of gonadotropin releasing hormone (GnRH) (Dhillo et al., 2005a; 2007b). Beck and Welch, (2010) reported that *KiSS-1* gene based treatments would be theoretically straightforward as long as the metastatic tumor cells express the *KiSS-1* receptor but most of the tumor cells does not express *KiSS-1* receptor therefore this could be a challenge for the researcher to develop a multiple cancer treatment with *KiSS-1* gene.

Conclusion

Collectively, in this review, there exist strong records to substantiated the molecular and functional features of *KiSS-1* expression system in a variety of cancer models and these studies suggest that *KiSS-1*/GPR54 system may participate in fundamental role of tumor biology and metastatic process; therefore, targeting *KiSS-1*/GPR54 system may represent a novel therapeutic agents for the treatment of metastatic cancer patients. Further investigation is essential to elucidate the complex pathways regulated by the Kisspeptins and how these pathways integrate in the suppression of metastasis across a range of cancers.

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References

Babwah AV, Pampillo M, Min L, et al (2012). Single-cell analyses reveal that *KiSS1*-R-expressing cells undergo sustained *Kiss*peptin-induced signaling that is dependent upon an influx of extracellular Ca_2^+. *Endocrinol*, **153**, 5875-87.

Bari R, Zhang YH, Zhang F, et al (2009). Transmembrane interactions are needed for *KAI1*/CD82-mediated suppression of cancer invasion and metastasis. *Am J Pathol*, **174**, 647-60.

Beck BH, Welch DR (2010). The *KiSS-1* metastasis suppressor:

a good night *KiSS* for disseminated cancer cells. *Eur J Cancer*, **46**, 1283-9.

Bilban M, Ghaffari-Tabrizi N, Hintermann E, et al (2004). Kisspeptin-10, a *KiSS-1*/metastin-derived decapeptide, is a physiological invasion inhibitor of primary human trophoblasts. *J Cell Sci*, **117**, 1319-28.

Dhar DK, Naora H, Kubota H, et al (2004). Downregulation of *KiSS-1* expression is responsible for tumor invasion and worse prognosis in gastriccarcinoma. *Int J Cancer*, **10**, 868-72.

Dhillo WS, Chaudhri OB, Patterson M, et al (2005a). Kisspeptin-54 stimulates the hypothalamic-pituitary gonadal axis in human males. *J Clin Endocrin Metab*, **90**, 6609-15.

Dhillo WS, Chaudhri OB, Thompson EL, et al (2007b). Kisspeptin-54 stimulates gonadotropin release most potently during the preovulatory phase of the menstrual cycle in women. *J Clin Endocrin Metab*, **92**, 3958-66.

Funes S, Hedrick JA, Vassileva G, et al (2003).The *KiSS-1* receptor GPR54 is essential for the development of the murine reproductive system. *Biochem Biophys Res Commun*, **26**, 1357-63.

Gao G, Liu L, Zou X, Chen W (2007). Expression of *KiSS-1*, metalloproteinase-9, nuclear fator-kappaBp56 in ovarian tumor. *Zhongua Fu Chan Ke Za Zhi*, **42**, 34-8.

Goldberg SF, Miele ME, Hatta N, et al (2003). Melanoma metastasis suppression by chromosome 6: evidence for a pathway regulated by CRSP3 and TXNIP. *Cancer Res*, **63**, 432-40.

Hameed S, Jayasena CN, Dhillo WS (2011). *Kiss*peptin and fertility. *J Endocrinol*, **208**, 97-105.

Hanahan D, Weinberg RA. (2011). Hallmarks of cancer: the next generation. *Cell*, **144**, 646-74.

Hata K, Dhar DK, Watanabe Y, et al (2007). Expression of metastin and a G-protein-coupled receptor (AXOR12) in epithelial ovarian cancer. *Eur J Cancer*, **43**, 1452-9.

Hunter KW, Crawford NPS, Alsarraj J (2008). Mechanisms of metastasis. *Breast Cancer Res*, **10**, 2.

Iiizumi M, Liu W, Pai SK, Furuta E, Watabe K (2008). Drug development against metastasis-related genes and their pathways: a rationale for cancer therapy. *Biochim Biophys Acta*, **1786**, 87-104.

Ikeguchi M, Hirooka Y, Kaibara N (2003). Quantitative reverse transcriptase polymerase chain reaction analysis for *KiSS-1* and orphan G-protein-coupled receptor (hOT7T175) gene expression in hepatocellular carcinoma. *J Cancer Res Clin Oncol*, **129**, 531-5.

Ikeguchi M, Yamaguchi K, Kaibara N (2004). 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*, **1**, 1379-83.

Khan N, Mukhtar H (2010). Cancer and metastasis: prevention and treatment by green tea. *Cancer Metastasis Rev*, **29**, 435-45.

Kostadima L, Pentheroudakis G, Pavlidis N (2007). The missing *KiSS* of life: transcriptional activity of the metastasis suppressor gene *KiSS-1* in early breast cancer. *Anticancer Res*, **27**, 2499-504.

Kotani M, Detheux M, Vandenbogaerde A, et al (2001). The metastasis suppressor gene *KiSS-1* encodes Kisspeptins, the natural ligands of the orphan G protein-coupled receptor GPR54. *J Biol Chem*, **276**, 34631-6.

Lee JH, Miele ME, Hicks DJ, et al (1996). *KiSS-1*, a novel human malignant melanoma metastasis-suppressor gene. *J Natl Cancer Inst*, **88**, 1731-7.

Li D, Yu W, Liu M (2009). Regulation of *KiSS-1* gene expression. *Peptides*, **30**, 130-8.

Li N, Wang HX, Zhang J, et al (2012). *KiSS-1* inhibits the proliferation and invasion of gastric carcinoma cells. *World J Gastroenterol*, **21**, 1827-33.

Liang S, Yang ZL (2007). Expression of *KiSS-1* mRNA in pancreatic ductal adenocarcinoma and non-cancerous pancreatic tissues in SD rats. *Zhong Nan Da Xue Xue Bao Yi Xue Ban*, **32**, 109-13.

Makri A, Pissimissis N, Lembessis P, Polychronakos C, Koutsilieris M (2008). The *Kiss*peptin (*KiSS-1*)/GPR54 system in cancerbiology. *Cancer Treat Rev*, **34**, 682-92.

Martin TA, Watkins G, Jiang WG (2005). *KiSS-1* expression in human breast cancer. *Clin Exp Metastas*, **22**, 503-11.

Moya P, Esteban S, Fernandez-Suarez A, et al (2013). *KiSS-1* methylation and protein expression patterns contribute to diagnostic and prognostic assessments in tissue specimens for colorectal cancer. *Tumour Biol*, **34**, 471-9.

Nash KT, Welch DR (2006). The *KiSS*1 metastasis suppressor: mechanistic insights and clinical utility. *Front Biosci*, **1**, 647-59.

Oakley AE, Clifton DK, Steiner RA (2009). *Kiss*peptin signaling in the brain. *Endocr Rev*, **30**, 713-43.

Ohtaki T, Shintani Y, Honda S, et al (2001). Metastasis suppressor gene *KiSS-1* encodes peptide ligand of a G-protein-coupled receptor. *Nature*, **411**, 613-7.

Olbrich T, Ziegler E, Türk G, et al (2010). *Kiss*peptin-10 inhibits bone-directed migration of GPR54-positive breast cancer cells: Evidence for a dose-window effect. *Gynecol Oncol*, **119**, 571-8.

Pineda R, Aguilar E, Pinilla L, Tena-Sempere M (2010). Physiological roles of the *Kiss*peptin/GPR54 system in the neuroendocrine control of reproduction. *Prog Brain Res*, **181**, 55-77.

Prabhu VV, Siddikuzzaman, Grace VM, Guruvayoorappan C (2012). Targeting tumor metastasis by regulating Nm23 gene expression. *Asian Pac J Cancer Prev*, **13**, 3539-48.

Ringel MD, Hardy E, Bernet VJ, et al (2002). Metastin receptor is over expressed in papillary thyroid cancer and activates MAPkinase in thyroid cancer cells. *J Clin Endocrinol Metab*, **87**, 2399.

Roa J, Tena-Sempere M (2007). *KiSS-1* system and reproduction: comparative aspects and roles in the control of female gonadotropic axis in mammals. *Gen Comp Endocrinol*, **153**, 132-40.

Roa J, Aguilar E, Dieguez C, Pinilla L, Tena-Sempere M (2008). New frontiers in Kisspeptin/GPR54 physiology as fundamental gatekeepers of reproductive function. *Front Neuroendocrinol*, **29**, 48-69.

Sanchez-Carbayo M, Capodieci P, Cordon-Cardo C (2003). Tumor suppressor role of *KiSS-1* in bladder cancer: loss of *KiSS-1* expression is associated with bladder cancer progression and clinical outcome. *Am J Pathol*, **162**, 609-17.

Sakthivel KM, Prabhu VV, Guruvayoorappan C (2012). WAVEs: a novel and promising weapon in the cancer therapy tool box. *Asian Pac J Cancer Prev*, **13**, 1719-22.

Schmid K, Wang X, Haitel A, et al (2007). *KiSS-1* overexpression as an independent prognostic marker in hepatocellular carcinoma: an immunohistochemical study. *Virchows Arch*, **450**, 143-9.

Shengbing Z, Feng LJ, Bin W, Lingyun G, Aimin H (2009). Expression of *KiSS-1* gene and its role in invasion and metastasis of human hepatocellular carcinoma. *Anat Rec (Hoboken)*, **292**, 1128-34.

Shevde LA, Welch DR (2003). Metastasis suppressor pathways - an evolving paradigm. *Cancer Lett*, **198**, 1-20.

Shirasaki F, Takata M, Hatta N, Takehara K (2001). Loss of expression of the metastasis suppressor gene *KiSS-1* during melanoma progression and its association with LOH of chromosome6q16.3-q23. *Cancer Res*, **15**, 7422-5.

Smith JT, Clifton DK, Steiner RA (2006). Regulation of the neuroendocrine reproductive axis by Kisspeptin-GPR54 signaling. *Reproduction*, **131**, 623-30.

Smith SC, Theodorescu D (2009). Learning therapeutic lessons from metastasis suppressor proteins. *Nat Rev Cancer*, **9**, 253-64

Stafford LJ, Vaidya KS, Welch DR (2008). Metastasis suppressors genes in cancer. *Int J Biochem Cell Biol*, **40**, 874-91.

Stafford LJ, Xia C, Ma W, Cai Y, Liu M (2002). Identification and characterization of mouse metastasis-suppressor *KiSS-1* and its G-protein coupled receptor. *Cancer Res*, **62**, 5399-404.

Stark AM, Tongers K, Maass N, et al (2005). Reduced metastasis-suppressor gene mRNA-expression in breast cancer brain metastases. *J Cancer Res Clin Oncol*, **131**, 191-8.

Stathatos N, Bourdeau I, Espinosa AV, et al (2005). *KiSS-1*/G protein-coupled receptor 54 metastasis suppressor pathway increases myocyte-enriched calcineurin interacting protein 1 expression and chronically inhibits calcineurin activity. *J Clin Endocr Metabol*, **90**, 5432-40.

Takeda T, Kikuchi E, Mikami S, et al (2012). Prognostic role of *KiSS-1* and possibility of therapeutic modality of metastin, the final peptide of the *KiSS-1* gene, in urothelial carcinoma. *Mol Cancer Ther*, **11**, 853-63.

Tena-Sempere M, Felip A, Gómez A, Zanuy S, Carrillo M (2012). Comparative insights of the *Kiss*peptin/*Kiss*peptin receptor system: lessons from non-mammalian vertebrates. *Gen Comp Endocrinol*, **175**, 234-43.

Thiolloy S, Rinker-Schaeffer CW (2011). Thinking outside the box: using metastasissuppressors as molecular tools. *Semin Cancer Biol*, **21**, 89-98.

Yoshioka K, Ohno Y, Horiguchi Y, et al (2008). Effects of a *KiSS-1* peptide, a metastasis suppressor gene, on the invasive ability of renal cell carcinoma cells through a modulation of a matrix metalloproteinase 2 expression. *Lif Sci*, **83**, 332-8.

Yu G, Chen Y, Ni C (2007). Reduced protein expression of metastasisrelated genes (nm23, *KiSS*1, *KAI1* and p53) in lymph node and liver metastases of gastric cancer. *Int J Exp Path*, **88**, 175-83.