JBUON 2018; 23(1): 79-84
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

KISS1 and KISS1R expression in gastric cancer

Ioannis D. Kostakis[1,2]*, George Agrogiannis[3]*, Aristeidis G. Vaiopoulos[1,2]*, Eleni Mylona[3], Efstratios Patsouris[3], Gregory Kouraklis[1], Michael Koutsilieris[2]

[1]Second Department of Propedeutic Surgery, "Laiko" General Hospital, National and Kapodistrian University of Athens, Medical School, Athens, Greece; [2]Department of Experimental Physiology, National and Kapodistrian University of Athens, Medical School, Athens, Greece; [3]First Department of Pathology, National and Kapodistrian University of Athens, Medical School, Athens, Greece

*These authors contributed equally to this work.

Summary

Purpose: *Kisspeptins, which are derived from the gene KISS1, supress tumor progression. We intended to investigate the production of KISS1 and its receptor (KISSR) in gastric cancer.*

Methods: *The expression of KISS1 and KISS1R in both normal and cancer tissue was examined with immunohistochemistry in tissue specimens of 40 cases of gastric adenocarcinoma.*

Results: *KISS1 expression in normal gastric mucosa was much higher than in malignant mucosa. KISS1 expression was higher in early stages (stage I or II) than in advanced stages (stage III or IV), in tumors with intestinal histological type than in those with diffuse histological type, in tumors without lymphovascular invasion than in those with and in cancers of older patients (≥70 years) than in younger patients. No significant differences were found regarding other clinicopathological parameters. There was no KISS1R expression in cancer tissues, while only low levels of KISS1R were detected in normal gastric epithelium.*

Conclusions: *KISS1 expression is decreased during carcinogenesis in gastric mucosa. More advanced tumors and more aggressive histological types produce lower KISS1 levels. In addition, no KISS1R is produced in malignant gastric epithelium, while KISS1R is only weakly expressed in normal gastric epithelium.*

Key words: *immunohistochemistry, gastric cancer, KISS1, KISS1R, kisspeptin*

Introduction

The direct product of *KISS1* gene is a protein consisted of 145 amino acids that undergoes cleavage into smaller peptides called kisppeptins, namely kisspeptin-10, kisspeptin-13, kisspeptin-14 and kisspeptin-54 (or metastin). Their receptor is a transmembrane protein that belongs to the Gq/11 family, the KISS1-derived peptide receptor (KISS1R) or G protein-coupled receptor 54 (GPR54) [1-12]. Kisspeptins stimulate GnRH release and trigger puberty onset and they are involved in the placenta formation by regulating the trophoblast invasion [2-7,10,11]. They also inhibit migration of malignant cells [1-12] and cell proliferation [1,3,4,6-10,12], suppress tissue invasion [1-10,12], angiogenesis [3,7,10] and promote apoptosis [3,5,7,10,11].

The role of kisspeptins has been investigated in many types of malignancies, such as thyroid, endometrial, ovarian, breast, hepatocellualr, pancreatic, esophageal, bladder and lung cancer, melanoma, osteosarcoma, pheochromocytoma and choriocarcinoma [1-7,10-12]. Regarding KISS1 production in gastric cancer, there are few studies. In this study, we have studied KISS1 and KISS1R expression in gastric cancer with immunohistochemistry.

Correspondence to: Ioannis D. Kostakis, MD, MSc, PhD. 27 Achridos Str, Kato Patissia, 11144 Athens, Greece.
Tel: +30 210 2230855, Fax: +30 210 3813822, E-mail: i.d.kostakis@gmail.com
Received: 18/07/2017; Accepted: 02/08/2017

Methods

Patients and tissue samples

Included were 40 patients with gastric adenocarcinoma (18 women and 22 men, mean age: 64.4 years, range: 32-85 years) who had been subjected to gastrectomy in our department in a period of 56 months. Exclusion criteria were the presence of another type of gastric cancer, history of previous cancer (either gastric adenocarcinoma or another type) and the administration of neoadjuvant treatment. Two blocks were obtained, fixed with formalin and embedded in paraffin for each patient, one containing normal gastric tissue and the other one containing malignant gastric tissue. Slides from each block were cut, examined after staining with hematoxylin and eosin and processed for immunohistochemical analysis.

The classification of cancers was done according to the guidelines of World Health Organization [13] and their staging according to the 7th edition of TNM classification of malignant tumors of UICC (International Union Against Cancer) [14]. Table 1 shows patient and disease characteristics.

This study was approved by the Ethics Committee of "Laiko" General Hospital (date: June 11th, 2010, reference number: ES 344) and conformed to the Helsinki Declaration of 1975, as revised in 2000.

Immunohistochemistry

The fixation of surgical specimens was done with 10% formalin solution. A series of alcohols, xylene and paraffin was applied on the tissue samples within the first 24 hrs. Four μm thick sections were cut from the blocks and xylene was used for deparaffinization and a graded series of alcohols for rehydration. Antigens were retrieved by applying a citrate buffer with pH 6.0 at 96°C for 30 min in a microwave oven. Afterwards, 3% hydrogen peroxide was added for 5 min and then a serum-free protein block (Dako, Glostrup, Denmark) for 30 min. Overnight incubation of the slides at 4°C followed using a rabbit polyclonal antibody against human KISS1 (SC-15400, Santa Cruz Biotechnology, Santa Cruz, CA, USA) or a goat polyclonal antibody against human KISS1R (SC-48220, Santa Cruz Biotechnology, Santa Cruz, CA, USA), in a dilution of 1:100 using serum-free protein block. Drops of Link, Streptavidin and 3,3´diaminobenzidine (DAB) were applied on the slides on the next day for 30 min each, consecutively. Subsequently, hematoxylin was added for 5 min and the slides were immersed 10 times in a wash bath. Application of a wash solution followed all the previously described steps. Finally, an aqueous-based medium was used for mounting and coverslipping the slides. Human placenta was used as positive control, whereas the primary antibody was omitted for negative control.

Image analysis

One month following immunohistochemistry, image analysis was performed. We selected 4-6 representative optical fields of gastric mucosa from each slide (normal or malignant) that were photographed under 400x magnification with a digital camera (Nikon DS-2 MW, Nikon, Tokyo, Japan) that was connected with a light microscope (Nikon Eclipse 80i, Nikon, Tokyo, Japan). The obtained images were saved in the form of JPEG (Joint Photographic Experts Group) files and analyzed with the software Image Pro Plus 5.1 (Media Cybernetics, Bethesda, MD, USA). Concerning DAB staining, brown color indicated presence of KISS1 or KISS1R expression, whereas blue color of hematoxylin counterstain indicated its absence. Homogeneous intensity of DAB staining was detected in all sections of

Table 1. Patient and disease characteristics

Parameters	*n*
Gender	
Male	22
Female	18
Age, years	
<70	23
≥70	17
Maximum tumor diameter (cm)	
<4	19
≥4	21
Stage	
I	8
II	10
III	16
IV	6
T	
T1	4
T2	6
T3	17
T4	13
N	
N0	13
N1	9
N2	5
N3	13
M	
M0	34
M1	6
Grade	
High grade	24
Low grade	16
Lauren's classification	
Diffuse type	17
Intestinal type	23
Lymphovascular invasion	
No	21
Yes	19
Perineural invasion	
No	32
Yes	8

T:primary tumor infiltration, N:infiltrated regional lymph nodes, M:distant metastasis

normal gastric mucosa, whereas homogeneous or heterogeneous intensity of DAB staining was detected in sections of cancer gastric mucosa. Four optical fields (400x magnification) were chosen when there was homogeneous intensity and 6 when there was heterogeneous intensity. The average levels of intensity of brown color [on a linear scale from 0 (for black) to 255 (for white)] and the average percentage of extent of brown color were taken into consideration for each slide.

The following equation for estimating KISS1 and KISS1R expression was used:

> Protein expression= (255–average intensity levels of brown color) x average percentage of extent of brown color.

The mean value of intensity levels and the mean value of percentages of extent of brown color for the selected optical fields were the average values for each slide.

Statistics

The normality of data distribution was assessed with the Shapiro-Wilk test. The t-test or the Mann-Whitney U test were applied for the comparisons between two groups in case of normal or not normal data distribution, respectively. Analysis of variance (ANOVA) with the Bonferroni correction was used when three or more groups were compared. The Spearman's rank correlation coefficient was used for the estimation of correlations between two quantitative variables. All the tests were two-tailed. Results were considered significant if $p<0.05$. The SPSS software (version 22.0, IBM Corporation, Armonk, NY, USA) was used for the statistical analyses.

Results

Normal versus malignant gastric mucosa in regards to KISS1 expression

KISS1 was found in the cytoplasm of the gastric epithelial cells and several stromal cells (Figure 1). Normal gastric mucosa produced much more KISS1 than malignant mucosa in each patient ($p<0.00001$) (Figure 1).

KISS1 expression in normal gastric mucosa

There were no significant differences between male and female patients ($p=0.623$) or older (≥70 years) and younger (<70 years) patients ($p=0.988$) regarding KISS1 levels in normal gastric mucosa.

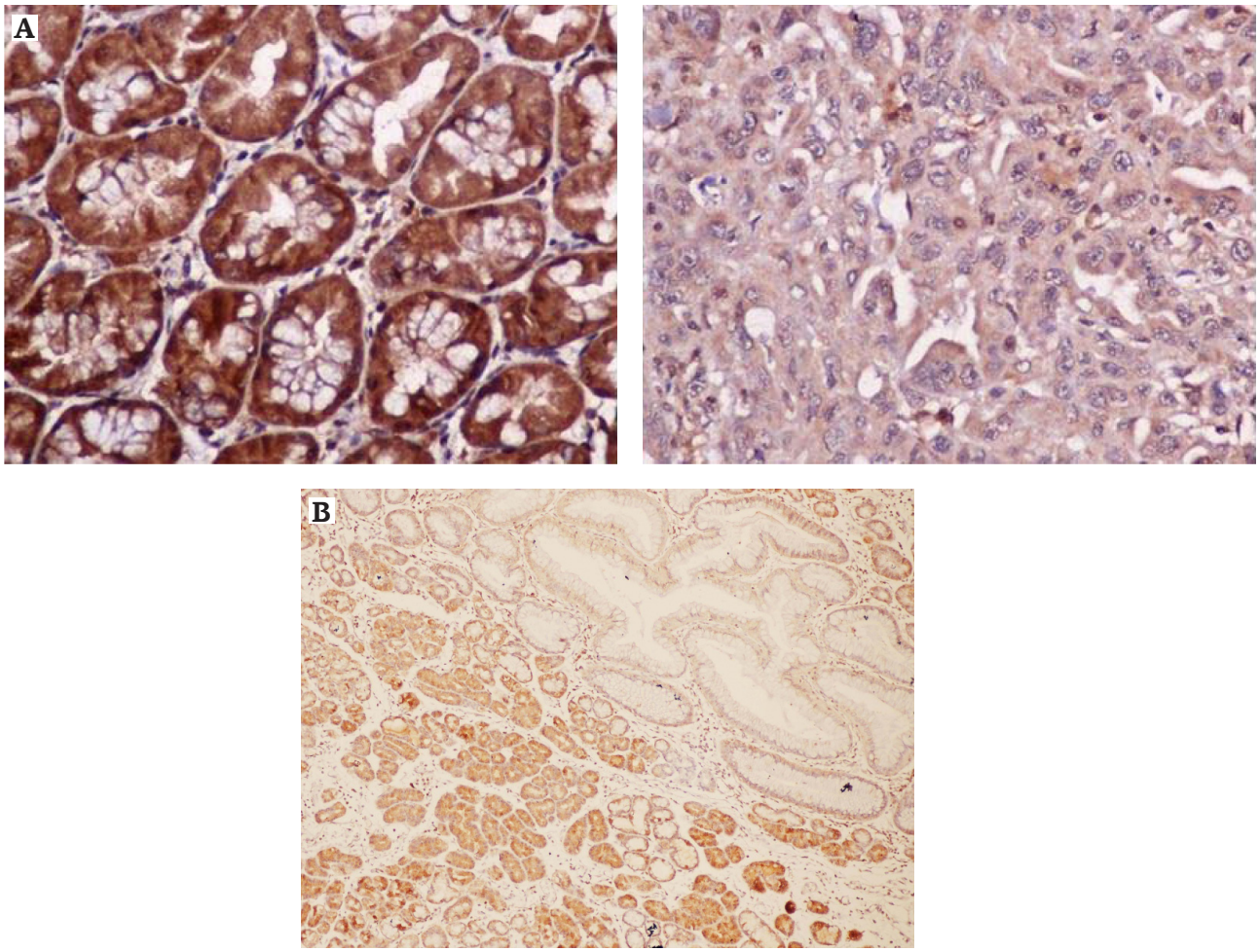

Figure 1. 3,3´diaminobenzidine (DAB) staining to show KISS1 production in normal gastric mucosa (**A:** left figure, **B:** lower left part) and gastric cancer (**A:** right figure, **B:** upper right part). DAB staining is weaker for gastric cancer (**A:** original magnification x400, **B:** original magnification x100).

KISS1 expression in gastric adenocarcinoma

KISS1 levels were higher in tumors from older (≥70 years) than from younger patients (<70 years) (p=0.015), while there were no significant differences when male and female patients were compared (p=0.59). Furthermore, KISS1 production was greater in early cancers (stages I/II) than in advanced cancers (stages III/IV) (p=0.043), in intestinal type tumors than in diffuse ones (p=0.03) (Figure 2) and in cases without lymphovascular invasion than in those with (p=0.042). On the contrary, no significant differences concerning KISS1 expression in malignant tissue were found between smaller (<4 cm in diameter) and larger (≥4 cm in diameter) tumors (p=0.638) or between low grade and high grade cancers (p=0.11). Moreover, the extent of the infiltration of the primary tumor (T) (p=0.532), the presence of infiltrated lymph nodes (p=0.273) or their number (N) (p=0.662), the presence of distant metastases (M) (p=0.054) and the presence of perineural invasion (p=0.055) seemed not to influence significantly KISS1 levels in malignant tissue.

Difference between normal and malignant tissue in regards to KISS1 expression

The difference between normal and malignant tissue in regards to KISS1 expression seemed not to be significantly affected by age (≥70 *vs.* <70 years) (p=0.22), gender (p=0.519), tumor size (≥4 *vs.* <4 cm in diameter) (p=0.936), stage of cancer (p=0.445), T (p=0.748), N (p=0.719), M (p=0.925), tumor grade (high grade *vs.* low grade) (p=0.479), histological type (intestinal *vs.* diffuse) (p=0.285), presence of lymphovascular invasiosn (p=0.995) and presence of perineural invasion (p=0.241).

KISS1R expression

No KISS1R production was found in malignant gastric cells, whereas only weak expression of KISS1R was found in the basal layer of normal gastric epithelium (Figure 3).

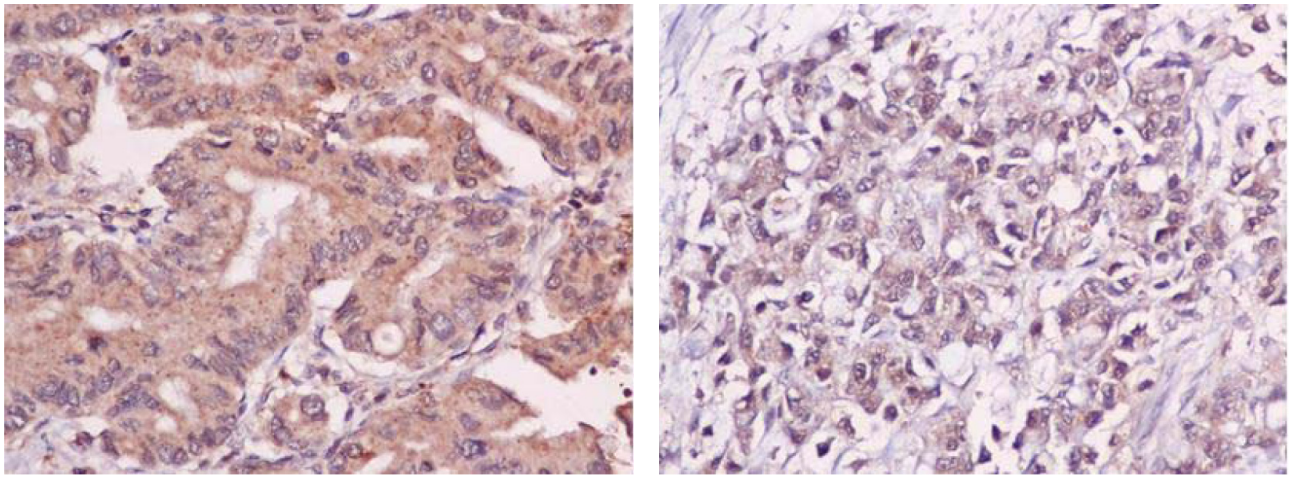

Figure 2. 3,3´diaminobenzidine (DAB) staining to show KISS1 expression in intestinal histological type (left) and diffuse histological type (right). DAB staining is stronger in intestinal histological type (original magnification x400).

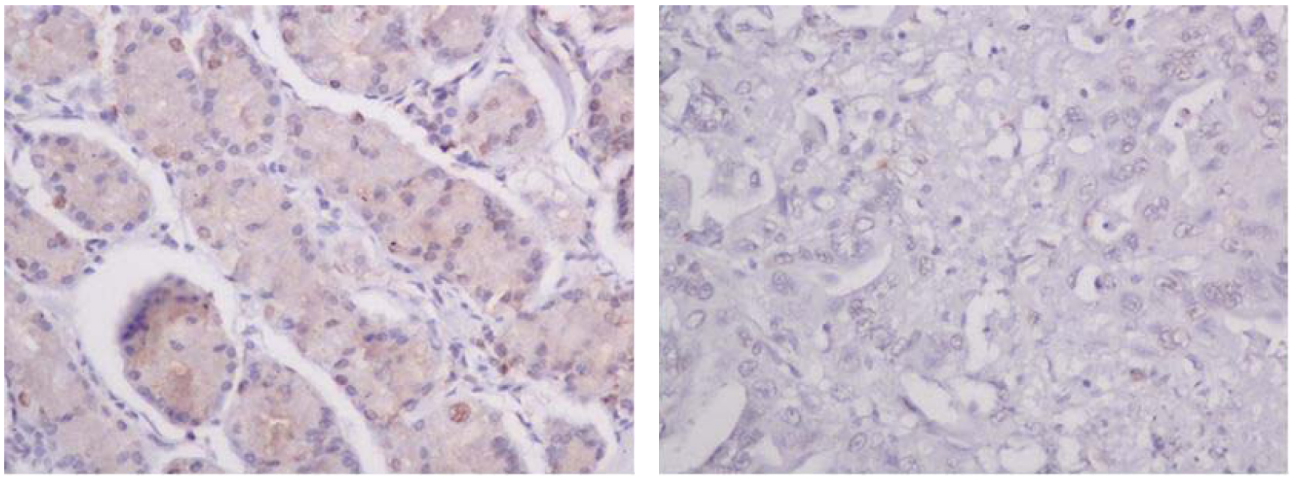

Figure 3. Low KISS1R production in normal gastric mucosa (left) and absence of KISS1R production in gastric cancer (right) (original magnification x400, 3,3´diaminobenzidine (DAB) staining).

Discussion

This study demonstrated that KISS1 levels are much higher in the normal gastric mucosa as compared with the malignant one. It was also found that gastric cancers with more advanced stage (stages III and IV), lymphovascular invasion and/or diffuse histological type, produce less KISS1 than gastric cancers with earlier stage (stages I and II), intestinal histological type and/or without lymphovascular invasion. In addition, gastric cancers in older patients (≥70 years) seem to express higher KISS1 levels than in younger patients (<70 years).

There are a few studies that have examined KISS1 expression in gastric cancer. Dhar et al. [15] showed that low KISS1 expression in gastic cancer is associated with distant metastasis, venous invasion, tumor recurrence and worse disease-free and overall survival. Furthermore, Guan-Zhen et al. [16] studied the immunohistochemical staining for KISS1 in primary gastric cancers and metastases in lymph nodes and the liver and reported that metastatic lesions produce less KISS1 in comparison with primary tumors. Yao et al. [17] used *in situ* hybridization for the detection of KISS1 expression in gastric cancers and pericancerous tissues and observed that gastric cancers had lower KISS1 levels than pericancerous tissues. They also found that malignant tumors with deeper infiltration (T3 or T4), second or third group lymph node metastasis and distant metastasis produced less KISS1 than malignant tumors with less infiltration depth (T1 or T2), without or with only first group lymph node metastasis and without distant metastasis. Moreover, Wang et al. [18] reported that the immunohistochemical staining for KISS1 was less intense in gastric cardia cancers with lymph node metastasis and advanced stage, but there was no correlation with the differentiation of malignant tissue. Zheng et al. [19] also pointed out an aberrant expression of KISS1 in association with lymph node metastasis. On the other hand, Ergen et al. [20] showed that kisspeptin-54 levels were higher in cases of gastric cancer as compared with healthy controls, but without association with any clinicopathological parameter. Regarding some *in vitro* studies in gastric cancer cell lines, Yamashita et al. [21] detected methylation of *KISS1* and *KISS1R* genes and Lee et al. [22] and Li et al. [23] noticed that KISS1 suppressed metalloproteinase-9 expression, inhibiting the proliferation and invasion of malignant gastric cells by activating the p38 MAP kinase signaling pathway.

In this study, we found that KISS1 levels were much higher in normal gastric mucosa than in gastric cancer, meaning that normal gastric epithelial cells produce much more KISS1 than malignant cells. This leads to the conclusion that KISS1 expression is downregulated during the malignant transformation of gastric mucosa. This conforms to the already known tumor suppressive actions of kisspeptins. Furthermore, we showed that more advanced gastric cancers (stages III and IV) and gastric cancers with the diffuse histological type, which are more aggressive and have worse prognosis than the intestinal histological type [24], as well as those tumors with lymphovascular infiltration, which are those ready to metastasize, produce less KISS1 than the others. These findings suggest that, as gastric cancer progresses, and especially in its more aggressive types, KISS1 expression is downregulated, leading to enhanced infiltrative and metastatic potential of malignant cells. Finally, the fact that no significant associations were detected concerning the difference of KISS1 production between normal gastric epithelium and gastric cancer possibly implies that the former levels of KISS1 production in normal gastric mucosa from which gastric cancer originates do not affect its expression by malignant cells as the tumor progresses.

Regarding KISS1R, no expression was detected in malignant gastric cells, whereas only a weak expression of KISS1R was found in the basal layer of normal gastric epithelium. These findings may suggest that either there is another not identified receptor of kisspeptins in gastric mucosa or that kisspeptins are produced in the stomach in order to act in other sites, as for example the liver, which produces both KISS1 and KISS1R [2-4,6-8,11], receives blood from the gastrointestinal tract via the portal circulation and is the most frequent and usually the first metastatic site of malignancies of the gastrointestinal tract [25].

Certain limitations should be acknowledged in this study. First, the number of participants was relatively small (n=40). Moreover, only one kind of samples, formalin-fixed, paraffin-embedded tissues, was used. KISS1 and KISS1R levels might also be assessed in fresh tissues and peripheral blood and correlated with their levels in fixed tissues. Furthermore, only one method, immunohistochemistry, was used for the assessment of KISS1 and KISS1R expression. Other methods, such as reverse transcriptase-polymerase chain reaction (RT-PCR) in fresh tissues and enzyme-linked immunosorbent assay (ELISA) in peripheral blood, might also be used for this purpose. Finally, KISS1 and KISS1R levels might also be estimated in metastatic sites, either nodal or distant, apart from the primary sites. Despite these limitations,

this study provides a reliable assessment of KISS1 and KISS1R production in gastric cancer.

In conclusion, the expression of KISS1 is decreased during carcinogenesis in gastric mucosa, resulting in reduced KISS1 production by malignant gastric cells when compared with normal ones. Moreover, KISS1 expression is further reduced as gastric cancer progresses, and especially in its more aggressive types, and therefore more advanced tumors and more aggressive histological types produce lower KISS1 levels than earlier stage tumors and less aggressive histological types. Thus, KISS1 may be used as a marker of advanced and/or more aggressive type of gastric cancer.

Acknowledgements

The authors would like to thank Maria Kemerli for her excellent technical assistance.

Conflict of interests

The authors declare no conflict of interests.

References

1. Beck BH, Welch DR. The KISS1 metastasis suppressor: a good night kiss for disseminated cancer cells. Eur J Cancer 2010;46:1283-9.
2. Li D, Yu W, Liu M. Regulation of KiSS1 gene expression. Peptides 2009;30:130-8.
3. Makri A, Pissimissis N, Lembessis P et al. The kisspeptin (KiSS-1)/GPR54 system in cancer biology. Cancer Treat Rev 2008;34:682-92.
4. Colledge WH. GPR54 and kisspeptins. Results Probl Cell Differ 2008;46:117-43.
5. Mead EJ, Maguire JJ, Kuc RE et al. Kisspeptins: a multifunctional peptide system with a role in reproduction, cancer and the cardiovascular system. Br J Pharmacol 2007;151:1143-53.
6. Nash KT, Welch DR. The KISS1 metastasis suppressor: mechanistic insights and clinical utility. Front Biosci 2006;11:647-59.
7. Murphy KG. Kisspeptins: regulators of metastasis and the hypothalamic-pituitary-gonadal axis. J Neuroendocrinol 2005;17:519-25.
8. Harms JF, Welch DR, Miele ME. KISS1 metastasis suppression and emergent pathways. Clin Exp Metastasis 2003;20:11-8.
9. Castaño JP, Martínez-Fuentes AJ, Gutiérrez-Pascual E et al. Intracellular signaling pathways activated by kisspeptins through GPR54: do multiple signals underlie function diversity? Peptides 2009;30:10-5.
10. Cho SG, Li D, Tan K et al. KiSS1 and its G-protein-coupled receptor GPR54 in cancer development and metastasis. Cancer Metastasis Rev 2012;31:585-91.
11. Kirby HR, Maguire JJ, Colledge WH et al. International Union of Basic and Clinical Pharmacology. LXXVII. Kisspeptin receptor nomenclature, distribution, and function. Pharmacol Rev 2010;62:565-78.
12. Ji K, Ye L, Mason MD et al. The Kiss-1/Kiss-1R complex as a negative regulator of cell motility and cancer metastasis (Review). Int J Mol Med 2013;32:747-54.
13. Bosman FT. WHO classification of tumours of the digestive system (4th Edn). World Health Organization. International Agency for Research on Cancer. IARC Press, Lyon, France, 2010.
14. Sobin LH, Gospodarowicz MK, Wittekind Ch. TNM classification of malignant tumours (7th Edn). International Union against Cancer; Hoboken, NJ: Wiley-Blackwell, Chichester, West Sussex, England, UK, 2010.
15. Dhar DK, Naora H, Kubota H et al. Downregulation of KiSS-1 expression is responsible for tumor invasion and worse prognosis in gastric carcinoma. Int J Cancer 2004;111:868-72.
16. Guan-Zhen Y, Ying C, Can-Rong N et al. Reduced protein expression of metastasis-related genes (nm23, KISS1, KAI1 and p53) in lymph node and liver metastases of gastric cancer. Int J Exp Pathol 2007;88:175-83.
17. Yao HL, Yang ZL, Li YG et al. In situ hybridization study on the expression of Kiss-1 and KAI-1 metastasis suppressor genes in gastric cancer. Zhonghua Wei Chang Wai Ke Za Zhi 2007;10:274-7.
18. Wang ZH, Feng XS, Wang GP et al. Expression and clinical significance of KiSS-1 and E-cadherin in gastric cardia carcinoma. Zhonghua Wei Chang Wai Ke Za Zhi 2007;10:380-2.
19. Zheng HC, Yu AM, Xin Y. Aberrant expression of Kiss-1 and matrix metalloproteinase-9 are closely linked to lymph node metastasis of gastric cancer. Chin Med Sci J 2008;23:63-4.
20. Ergen A, Canbay E, Bugra D et al. Plasma Kisspeptin-54 levels in gastric cancer patients. Int J Surg 2012;10:551-4.
21. Yamashita S, Tsujino Y, Moriguchi K et al. Chemical genomic screening for methylation-silenced genes in gastric cancer cell lines using 5-aza-2'-deoxycytidine treatment and oligonucleotide microarray. Cancer Sci 2006;97:64-71.
22. Lee KH, Kim JR. Kiss-1 suppresses MMP-9 expression by activating p38 MAP kinase in human stomach cancer. Oncol Res 2009;18:107-16.
23. Li N, Wang HX, Zhang J et al. KISS-1 inhibits the proliferation and invasion of gastric carcinoma cells. World J Gastroenterol 2012;18:1827-33.
24. Dicken BJ, Bigam DL, Cass C et al. Gastric adenocarcinoma: review and considerations for future directions. Ann Surg 2005;241:27-39.
25. Zeuzem S. Gut-liver axis. Int J Colorectal Dis 2000;15:59-82.