JBUON 2018; 23(3): 598-603
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

KISS1 and KISS1R expression in primary and metastatic colorectal cancer

Spyridon Matthaiou[1]*, Ioannis D. Kostakis[2]*, Stylianos Kykalos[2]*, Nikolaos Machairas[2], Eleftherios Spartalis[2], Evangelia Ntikoudi[2], Maria Lambropoulou[3], Anastasios Karayiannakis[1]

[1]Second Department of Surgery, Alexandroupolis University Hospital, Democritus University of Thrace, Medical School, Alexandroupolis, Greece; [2]Second Department of Propaedeutic Surgery, "Laiko" General Hospital, National and Kapodistrian University of Athens, Medical School, Athens, Greece; [3]Laboratory of Histology-Embryology, Democritus University of Thrace, Medical School, Alexandroupolis, Greece

*These authors contributed equally to this work.

Summary

Purpose: *Kisspeptins are produced by the KISS1 gene and have tumor-suppressing and anti-metastatic properties. Our aim was to study the expression of KISS1 and its receptor, KISS1R, in colorectal cancer.*

Methods: *KISS1 and KISS1R expression was detected using immunohistochemistry in malignant tissue samples from 66 patients (34 men, 32 women) with colorectal adenocarcinoma. In total, 74 tumor samples were studied, 57 samples from primary tumors and 17 samples from liver metastases. KISS1 and KISS1R levels were associated with various clinicopathological parameters and survival data.*

Results: *KISS1 expression was higher in primary tumors with advanced stage (III or IV) and in those with infiltrated lymph nodes. KISS1R expression was higher in primary tumors with distant metastases. No significant differences were detected between primary and metastatic tumors regarding KISS1 and KISS1R levels. Furthermore, patients with high KISS1R levels had longer overall survival.*

Conclusions: *KISS1 and KISS1R expression is higher in advanced colorectal cancers and high KISS1R levels are associated with better prognosis in colorectal cancer.*

Key words: *colorectal cancer, immunohistochemistry, KISS1, KISS1R, kisspeptin, large intestine*

Introduction

Kisspeptins are four peptides, namely kisspeptin-10, kisspeptin-13, kisspeptin-14 and kisspeptin-54 (or metastin), which are the products of the cleavage of a premature KISS1 protein, consisted of 145 amino acids. Their receptor is a G protein-coupled transmembrane receptor, named KISS1-derived peptide receptor (KISS1R) or G protein-coupled receptor 54 (GPR54) or AXOR12 [1-13]. Apart from their role in the stimulation of GnRH release, the onset of puberty and the placenta formation [2-7, 10-13], kisspeptins are involved in malignant transformation and metastatic potential of cancer cells [1-13]. In particular, it has been found that they inhibit cell migration and metastasis [1-11] by suppressing angiogenesis [3,7,10] and reducing the infiltration of underlying tissues [1-10]. They are also implicated in the suppression of cell proliferation [1,3,4,6-10] and the induction of apoptosis [3,5,7,10,11]. Since kisspeptins were firstly studied in melanoma, they and their recep-

Correspondence to: Ioannis D. Kostakis, MD, MSc, PhD. 27 Achridos Street, Kato Patissia, 11144 Athens, Greece.
Tel: +30 210 2288698, Fax: +30 210 3813822, E-mail: i.d.kostakis@gmail.com
Received: 15/11/2017; Accepted: 11/12/2017

tor have also been studied in several other types of malignancies, such as hepatocellular, pancreatic, gastric, esophageal, ovarian, endometrial, breast, prostate, bladder, thyroid and lung cancer, pheochromocytoma, osteosarcoma and choriocarcinoma, with some studies claiming that KISS1 expression is decreased in malignant cells and as cancer progresses, while others argue for the opposite [1-7,10-13]. As far as the role of KISS1 and KISS1R in colorectal cancer is concerned, only a few studies have been conducted. Our aim was to further investigate this issue in this type of malignancy.

Methods

Patients and tissue samples

Sixty-six patients (34 men and 32 women) with colorectal adenocarcinoma who had been operated in our department were included in this study. Fifty-seven patients had undergone complete resection of their primary tumor. Eight out of them had also undergone liver resection due to synchronous liver metastases. There were also 9 patients who had undergone liver resection due to metachronous liver metastases. Thus, there were 74 tumor samples in total, 57 samples from primary tumors and 17 samples from liver metastases. A formalin-fixed, paraffin-embedded archival block was obtained for each tumor sample. Staging was performed using the 8th edition of the TNM Classification of Malignant Tumors according to the International Union Against Cancer (UICC) [14]. This study conforms to the Declaration of Helsinki and the guidelines of the Ethical Committee of our institution. All patients gave oral informed consent to participate in this study. Patients' clinicopathological data are listed in Table 1.

Immunohistochemistry

Four μm thick sections were cut from each formalin-fixed, paraffin-embedded block. Paraffin was removed using xylene and a graded series of alcohols was used for rehydration. For identification of KISS1 expression, a rabbit polyclonal antibody against KISS1 (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) was applied with a dilution of 1:200 for 30 min, after an EDTA buffer solution (pH 8.0) had been applied for antigen retrieval for 30 min. For identification of KISS1R expression, a rabbit polyclonal antibody against KISS1R (Phoenix Pharmaceuticals, Inc., Burlingame, CA, USA) was applied with a dilution of 1:70 for 20 min, after a citrate buffer solution (pH 6.0) had been applied for antigen retrieval for 20 min. Afterwards, a biotinylated anti-rabbit antibody, streptavidin and 3,3´-diaminobenzidine (DAB) were consecutively added for 30 min each. In the end, hematoxylin was added for 30 min. All the steps were followed by rinse with a wash solution. The primary antibody was omitted for negative controls. The immunohistochemical expression of KISS1 and KISS1R was evaluated as follows: 0: negative staining; 1: mild staining; 2: intermediate staining; 3: intense staining. The expression was regarded as low when the immunohistochemistry score was 0 or 1 and high when the immunohistochemistry score was 2 or 3.

Statistics

Comparisons among groups with categorical variables were made using the Chi-square test and the Fisher's exact test when the samples were independent and the McNemar test when the samples were matched. Kaplan-Meier curves were used for the assessment of overall survival and the comparisons of survival among different groups were made using the log-rank test. Cox regression with the forward conditional method was used for multivariate survival analysis. All the tests were two-tailed. The level of statistical significance was set at p value less than 0.05.

Results

KISS1 expression in primary and metastatic tumors

Eighteen out of the 57 primary tumors (31.6%) showed low KISS1 expression, whereas 39 out of the 57 primary tumors (68.4%) showed high KISS1

Table 1. Clinicopathological data of patients with colorectal cancer (n=66)

Parameters	*Number of patients*
Gender	
Male	34
Female	32
Types of tissue specimens	
Primary tumor only	49
Primary tumor and liver metastasis	8
Liver metastasis only	9
Characteristics of primary tumors (n=57)	
Stage	
I	14
II	9
III	16
IV	18
Extent of primary tumor infiltration (T)	
T1	0
T2	21
T3	34
T4	2
Infiltrated regional lymph nodes (N)	
N0	29
N1/N2	28
Distant metastases (M)	
M0	39
M1	18
Histological grade	
G1	12
G2	33
G3	12

expression (Figure 1). Primary tumors of patients with stage III or IV disease demonstrated more often high KISS1 levels (27 out of 34; 79.4%) than patients with stage I or II disease (12 out of 23; 52.2%) (p=0.03). Moreover, high KISS1 levels were detected more frequently in primary tumors of patients with infiltrated lymph nodes (23 out of 28; 82.1%) than in patients without infiltrated lymph nodes (16 out of 29; 55.2%) (p=0.029). On the contrary, no significant associations were found between KISS1 expression in primary tumors and the direct extent of the primary tumor (p=0.709), the histological grade of the neoplasm (p=0.366) and the presence or the absence of distant metastases (p=0.1).

Eight out of the 17 metastatic tumors (47.1%) demonstrated low KISS1 expression, while 9 out of the 17 metastatic tumors (52.9%) demonstrated high KISS1 expression (Figure 2). When the primary tumors were compared with the metastatic tumors, no significant difference was observed in regards to the KISS1 expression (p=0.241). The same applied when only the 8 patients with specimens from both the primary tumor and liver metastasis were taken into account, since all the 8 primary tumors (100%) and 7 out of 8 liver metastases (87.5%) had high KISS1 levels (p=1).

KISS1R expression in primary and metastatic tumors

Thirty-five out of the 57 primary tumors (61.4%) showed low KISS1R expression, whereas 22 out of the 57 primary tumors (38.6%) showed high KISS1R expression (Figure 3). High KISS1R levels were observed more often in primary tumors of patients with distant metastases (stage IV disease) (12 out of 18; 66.7%) than in patients without distant metastases (stage I-III disease) (10 out of 39; 25.6%) (p=0.003). On the other hand, there were no significant associations between KISS1R expression in primary tumors and the direct extent of the primary tumor (p=0.08), the histological grade of the neoplasm (p=0.427) and the presence or the absence of infiltrated lymph nodes (p=0.233).

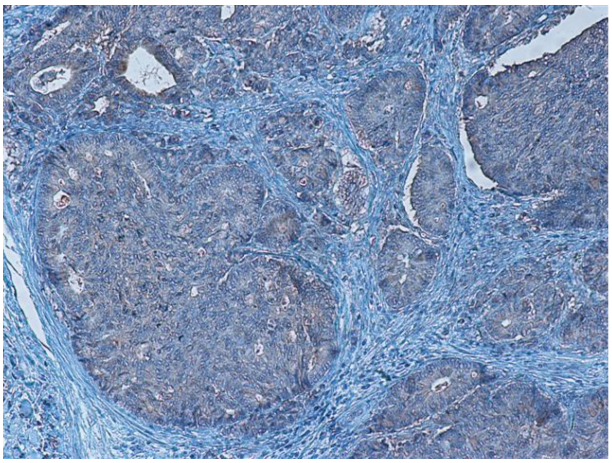

Figure 1. KISS1 expression in a primary tumor (original magnification ×200).

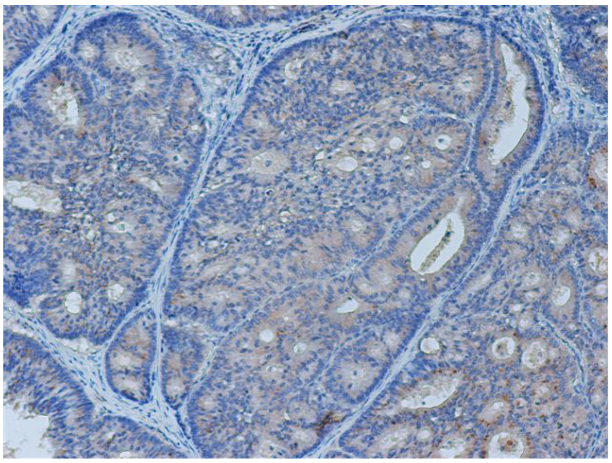

Figure 3. KISS1R expression in a primary tumor (original magnification ×200).

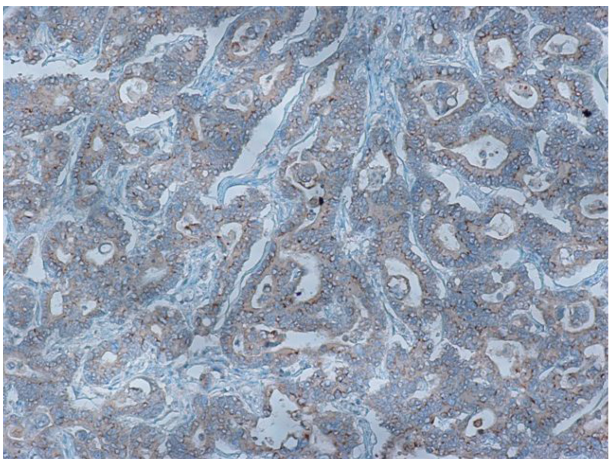

Figure 2. KISS1 expression in a metastatic tumor (original magnification ×200).

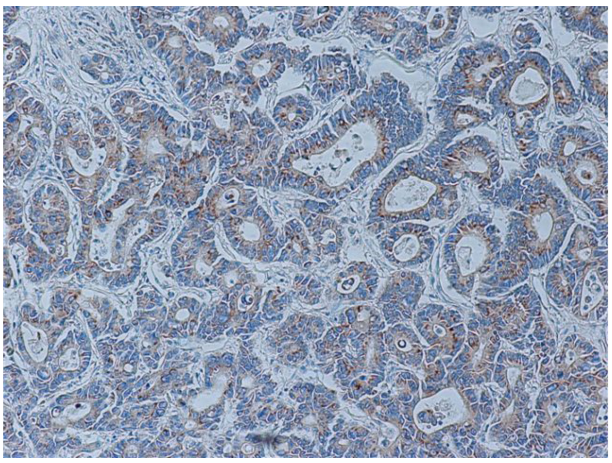

Figure 4. KISS1R expression in a metastatic tumor (original magnification ×200).

Seven out of the 17 metastatic tumors (41.2%) demonstrated low KISS1R expression, while 10 out of the 17 metastatic tumors (58.8%) demonstrated high KISS1R expression (Figure 4). When the primary tumors were compared with the metastatic tumors, no significant difference was observed in regards to the KISS1 expression (p=0.14). The same applied when only the 8 patients with specimens from both the primary tumor and liver metastasis were taken into account, since all the 8 primary tumors (100%) and all the 8 liver metastases (100%) had high KISS1R levels (p=1).

Survival analysis according to KISS1 and KISS1R expression

The mean surveillance period was 18.4 months (standard deviation 20.1) and the median surveillance period was 11 months (min-max: 0-61). There were available data for 31 patients who had undergone resection of their primary tumors regarding overall survival, 29 of whom (93.5%) had died during the surveillance period. There was no significant difference between patients with low and high KISS1 levels in the primary tumor concerning overall survival (p=0.297) (Figure 5). However, patients with high KISS1R expression in the primary tumor had longer overall survival than patients with low KISS1R expression in the primary tumor (low KISS1R expression: mean: 11.7 months, standard error (SE): 3.3, 95% CI: 5.1-18.2, median: 7 months; high KISS1R expression: mean: 37.7 months, SE: 8.2, 95% CI: 21.6-53.7, median: 53 months, p=0.005) (Figure 6). High KISS1R levels in the primary tumor remained an independent prognostic factor of better overall survival in the multivariate survival analysis (HR: 0.309, 95% CI: 0.126-0.757, B: -1.175, SE: 0.457, Wald: 6.596, p=0.01).

Discussion

Kisspeptins have been studied in several types of malignancies [1-7,10-13]. It has been found that kisspeptins inhibit cell migration and metastasis [1-11], suppress cell proliferation [1,3,4,6-10] and induce apoptosis [3,5,7,10,11]. A few studies have been conducted in several colorectal cancer cell lines supporting the already known role of kisspeptins [15-22]. In particular, treatment of SW480 cells with radiation resulted in increased KISS1 mRNA levels, decreased cell proliferation and higher rates of apoptosis [15]. In addition, the administration of azacytidine led to increase of KISS1 mRNA levels in SW480, RKO [16] and HCT116 cells [17]. Furthermore, KISS1 overexpression resulted in inhibition of cell proliferation, invasion and migration and induction of apoptosis in HCT116 cells [18,19], whereas the knockdown of KISS1 expression led to increased invasiveness and migration of HT115 cells [20]. Similarly, it has been observed that KISS1 inhibits cell proliferation, invasion and migration of HT29 [21] and LoVo cells [22].

However, the findings of clinical studies are contradictory, with most articles reporting results that support the tumor-suppressive properties of KISS1, while others mentioning opposite results. Liang et al. [23], Moya et al. [16] and Kostakis et al. [24, 25] detected higher KISS1 expression in normal large intestine than in colorectal cancer.

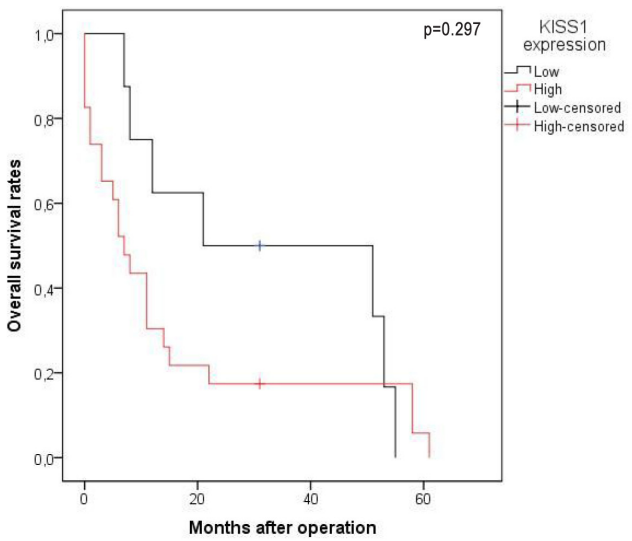

Figure 5. Overall survival according to KISS1 expression in primary tumors.

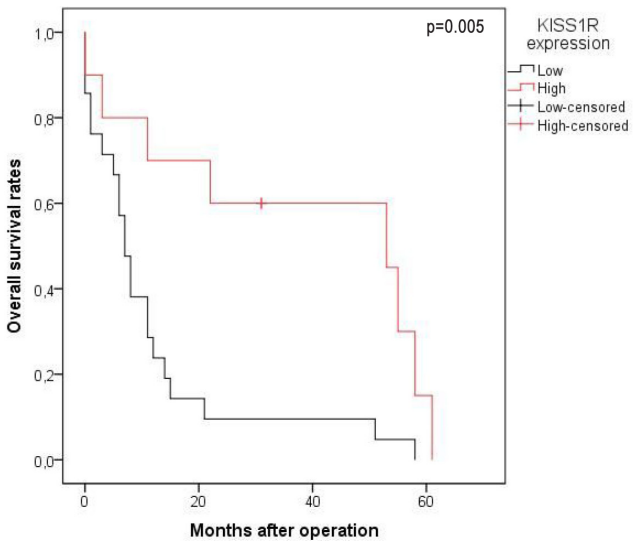

Figure 6. Overall survival according to KISS1R expression in primary tumors.

Similarly, Wang et al. [26] reported that KISS1 expression is also higher in tissues from benign than in tissues from malignant colorectal diseases. Moreover, lower KISS1 levels were observed in advanced colorectal cancers than in early ones by Liang et al. [23], Moya et al. [16], Okugawa et al. [27], Stathaki et al. [28] and Ji et al. [20] and they were associated with worse prognosis by Moya et al. [16], Okugawa et al. [27] and Zhu et al. [29]. On the contrary, Okugawa et al. [27] found no KISS1 expression and Stathaki et al. [28] found only low or moderate KISS1 expression in normal large intestine, while Kostakis et al. [24,25] detected higher KISS1 expression in advanced colorectal cancers, which was associated with worse prognosis. In addition, Canbay et al. [30] found elevated kisspeptin-54 plasma levels in patients with colorectal cancer in comparison with healthy controls and observed high kisspeptin-54 plasma levels more often in patients with infiltrated lymph nodes than in patients without. Finally, only three studies reported findings regarding KISS1R expression in colorectal cancer [20,25,29]. In particular, Ji et al. [20] observed higher KISS1R levels in normal than in malignant tissues, whereas Kostakis et al. [25] detected no KISS1R expression in normal or malignant tissues. Results concerning prognosis were also contradictory, with Ji et al. [20] reporting shorter survival, while Zhu et al. [29] reporting longer survival in patients with high KISS1R levels.

Our results contradict the already known tumor-suppressive properties of kisspeptins. We found that KISS1 and KISS1R levels are higher in advanced colorectal cancers and there is no actual difference in regards to their expression between primary and metastatic tumors. Nevertheless, the patients with high KISS1R expression have longer overall survival than the patients with low KISS1R expression. On the other hand, no significant associations were detected between KISS1 levels and overall survival. Our findings, as well as the findings of studies regarding hepatocellular, breast and ovarian cancers, in which KISS1 levels are higher in advanced disease stages, imply that kisspeptins may not possess only inhibitory properties. As Kostakis et al. [24,25] have previously suggested, kisspeptins may also possess properties that promote tumor progression in certain types of malignancies or alternatively, they may be activated in advanced disease stages, when malignant cells attempt to metastasize, as a defense mechanism against cell migration.

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, Polychronakos C, Koutsilieris M. 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, Davenport AP. 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, Vaudry H, Tena-Sempere M, Malagón MM. 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, Siwko SK, Liu M. 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, Davenport AP. 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, Jiang WG. 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. Prabhu VV, Sakthivel KM, Guruvayoorappan C. Kisspeptins (KiSS-1): essential players in suppressing tumor metastasis. Asian Pac J Cancer Prev 2013;14:6215-20.
14. Brierley JD, Gospodarowicz MK, Wittekind C (Eds). Colon and Rectum. In: TNM classification of malignant tumours. 8th edition. Wiley-Blackwell, Oxford, UK, 2016;73-6.

15. Chen SQ, Tu MM, Dai QB, Lin SY, Ke CL. Kiss-1 gene expression after radiation and its association with proliferation and apoptosis in colorectal cancer cells. Zhonghua Wei Chang Wai Ke Za Zhi 2012;15:508-11.
16. Moya P, Esteban S, Fernandez-Suarez A, Maestro M, Morente M, Sánchez-Carbayo M. KiSS-1 methylation and protein expression patterns contribute to diagnostic and prognostic assessments in tissue specimens for colorectal cancer. Tumour Biol 2013;34:471-9.
17. Chen SQ, Chen ZH, Lin SY, Dai QB, Fu LX, Chen RQ. KISS1 methylation and expression as predictors of disease progression in colorectal cancer patients. World J Gastroenterol 2014;20:10071-81.
18. Chen S, Su X, Gao J, Han H, Chen Z, Lin S. Suppression of Kiss-1 gene inhibits HCT116 human colorectal carcinoma cell migration in vitro via nuclear factor-κB signaling pathway. Nan Fang Yi Ke Da Xue Xue Bao 2015;35:1643-8.
19. Chen S, Chen W, Zhang X, Lin S, Chen Z. Overexpression of KiSS-1 reduces colorectal cancer cell invasion by downregulating MMP-9 via blocking PI3K/Akt/NF-κB signal pathway. Int J Oncol 2016;48:1391-8.
20. Ji K, Ye L, Ruge F, Hargest R, Mason MD, Jiang WG. Implication of metastasis suppressor gene, Kiss-1 and its receptor Kiss-1R in colorectal cancer. BMC Cancer 2014;14:723.
21. Zhang B, Li A, Zuo F et al. Recombinant Lactococcus lactis NZ9000 secretes a bioactive kisspeptin that inhibits proliferation and migration of human colon carcinoma HT-29 cells. Microb Cell Fact 2016;15:102.
22. Kim TH, Cho SG. Kisspeptin inhibits cancer growth and metastasis via activation of EIF2AK2. Mol Med Rep 2017;16:7585-90.
23. Liang QL, Chen XD, Wang SM et al. Expression of vascular endothelial growth factor and metastin in colorectal carcinoma. Nan Fang Yi Ke Da Xue Xue Bao 2007; 27:1584-7.
24. Kostakis ID, Agrogiannis G, Vaiopoulos AG et al. KISS1 expression in colorectal cancer. APMIS 2013;121:1004-10.
25. Kostakis ID, Agrogiannis G, Vaiopoulos AG et al. A clinicopathological analysis of KISS1 and KISS1R expression in colorectal cancer. APMIS 2015;123:629-37.
26. Wang W, Qi Y, Xu Q, Ren H. Expression and clinical significance of kisspeptin-1, matrix metalloproteinase-2 and vascular endothelial growth factor in tissue of colon cancer. Zhonghua Liu Xing Bing Xue Za Zhi 2016;37:415-7.
27. Okugawa Y, Inoue Y, Tanaka K et al. Loss of the metastasis suppressor gene KiSS1 is associated with lymph node metastasis and poor prognosis in human colorectal cancer. Oncol Rep 2013;30:1449-54.
28. Stathaki M, Armakolas A, Dimakakos A et al. Kisspeptin effect on endothelial monocyte activating polypeptide II (EMAP-II)-associated lymphocyte cell death and metastases in colorectal cancer patients. Mol Med 2014;20:80-92.
29. Zhu C, Takasu C, Morine Y et al. KISS1 associates with better outcome via inhibiting matrix metalloproteinase-9 in colorectal liver metastasis. Ann Surg Oncol 2015;22: S1516-S1523.
30. Canbay E, Ergen A, Bugra D et al. Kisspeptin-54 levels are increased in patients with colorectal cancer. World J Surg 2012;36:2218-24.