HOTAIR, *HOXC13*, and *HOXC10* Are Overexpressed in Gastric Cancer

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Abstract

Gastric cancer is concerned as the second leading cause of tumor-associated death worldwide after lung tumors and is specified as one of the most common malignant cancers. Given the increasing importance of *HOX* genes in gastric cancer research, this study was aimed at exploring the expression profile of *HOTAIR*, *HOXC13*, *HOXC10*, *HOXC13-AS*, and *HOXC-AS3* in gastric cancer. To achieve this goal, 30 pairs of tumor and normal margin samples were assessed for these genes expression analyses via qRT-PCR. result we found that, the *HOTAIR*, *HOXC13*, and *HOXC10* expression was significantly increased in cancer tissue samples in comparison with adjacent normal tissue samples, $P < 0.01$. Moreover, there were significant positive correlations between the expressions of genes studied: *HOXC-AS3* and *HOXC10* ($r = 0.52$, $P < 0.003$), *HOXC13-AS* and *HOXC13* ($r = 0.57$, $P < 0.001$), *HOTAIR* and *HOXC-AS3* ($r = 0.39$, $P < 0.03$), *HOTAIR* and *HOXC13-AS* ($r = 0.36$, $P < 0.05$). The *HOXC13* and *HOXC10* expression exhibited a significant relationship with both distant metastasis and perineural invasion (metastasis: $P < 0.014$, $r = 0.443$ and $P < 0.0041$, $r = 0.51$ perineural invasion: $P < 0.025$, $r = 0.41$ and $P < 0.0017$, $r = 0.55$). The *HOXC13-AS* displayed a significant relationship with the sex factor so that in females the expression of this gene was higher than males ($P < 0.030$, $r = 0.4$). Our findings suggest that the expression of *HOXC* genes and *HOTAIR* lncRNA increased through gastric tumor progression and thus they possibly participate in malignant transformation and gastric carcinoma metastasis.

Introduction

Gastric cancer is known as the second leading cause of tumor-associated death in the world after lung cancers (1). This cancer is histologically categorized into two types: intestinal and diffuse. Moreover, this is anatomically categorized into gastro-esophageal borderline and gastric adenocarcinoma (2). With the implementation of neoadjuvant therapy, chemotherapy, radiotherapy, and surgical procedures, the five-year survival of patients with primary gastric cancer can reach 94% (3). Despite these advances, this cancer is rarely diagnosed in the early step and thereby in most patients, the tumor has gone forward to an advanced step over the time of clinical manifestations (4). As an output of the metastasis and malignant invasion observed in later steps, the traditional techniques of chemotherapy, radiotherapy, and surgery are not as efficient as in the primary step, and the five-year survival rate is $< 22\%$ (3). At the moment, the key therapy for advanced gastric cancer encompasses a combination of immunotherapy, molecular targeted therapy, and chemo-radiotherapy (2). To explore the mechanisms underlying the gastric cancer pathogenesis and recognize more efficient markers anticipating response to treatment and prognosis, a variety of studies is required on all perspectives of the gastric cancer (1). Currently, numerous documents have emerged for the role of *HOX* genes and their lncRNAs in the progression and pathogenesis of gastric cancer through exploring signaling networks (5).

HOX genes play a role as a tumor suppressor or oncogene gene, depending on cellular context (6). In cancers, abnormal expression of these genes is the general mechanism of loss or gain of function (7). Mutation in the gene family *HOX* is rare but has been reported previously (6). For example, some of them behave as a gene coding tumor suppressor, which blocked the initiation of prostate tumor, and mutation

in the germline render an individual more sensitive to this disease (8). Given the importance of gene family *HOX*, a number of research and reviews covered this topic (5). In most cases, the authors suggested that *HOX* genes modulate epithelial to mesenchymal transition (EMT), proliferation, apoptosis, differentiation, and angiogenesis in a couple of cancers (5).

The capability of tumor cells to maintain their proliferation state is its most key feature, achieved by the keeping of growth signal through the paracrine/autocrine growth factor pathways (9). Non-cancer tissue solidly modulates the generation and release of growth-promoting signals, which in turn regulate division and growth to guarantee cell homeostasis and therefore retain regular function/structure (10). Tumor cell regulates these messages to control tumor growth, and long non-coding RNA (lncRNA) molecules participate in this process via modulating the growth factors (10). LncRNAs, as molecules with more than 200 nucleotides, account for more than 85% of non-coding RNAs and are the novel focus points in the area of tumor research (4). Regulatory lncRNAs such as *HOTAIR* mediate the anti-proliferative effect and thereby decrease gastric cancer invasion, therefore leading to therapeutic efficacies, and making it feasible to concern the application of long non-coding RNAs as a therapeutic target in gastric cancer (11).

To explore the role of *HOTAIR*, *HOXC13*, *HOXC10*, *HOXC13-AS*, and *HOXC-AS3* in the development of gastric tumor, we assessed the expression of these genes in gastric cancer and margin normal tissues.

Materials And Methods

Patients and tissue sample

Herein, a total of 30 cancer tissue samples along with their healthy adjacent tissue samples were prepared from the individuals who suffered from gastric cancer. The samples were from cancer institute, Tehran, Iran. Briefly, samples were provided from patients with gastric cancer following surgical procedures and maintained immediately in liquid nitrogen. The informed consent was recorded from all patients who participated in this study.

Total RNA extraction

To extract the total RNA, 100 mg of frozen tissue samples was crushed and then homogenized. The extraction of total RNA was done using TRIzol® Reagent (Invitrogen Co., United States). The RNA concentration and integrity were evaluated using the spectrophotometer Nano Drop™ 2000 (Invitrogen Co., United States) and the electrophoresis on a 1% agarose gel containing Safe Stain (Cinna Gen Co., United States), respectively. Extracted RNA was treated with DNaseI to remove DNA contaminations.

cDNA synthesis

We used TAKAR cDNA synthesis kit (Invitrogen Co., United States). The reverse transcription of RNA to cDNA was performed as the protocol described by manufacture. Briefly, 1 µl random hexamer sequences (100 µM) and 1 µl oligo (dT) primer (50 µM) were added to the 1µg extracted RNA, and then RNase free water was added up to total volume of 5 µl and incubated for 5 min at 60 °C. Then, 4 µl of 5X buffer and

1 µl of RT enzyme (1 U/µl) were added, and RNase free water was incorporated into an ultimate volume of 20 µl and incubated at 37 °C for 25 min. Finally, the reaction was incubated for 5 s at 80 °C to inactivate the RT enzyme and cDNA was maintained at -20 °C for further assessment.

Quantitative real-time PCR

For quantitative Real-Time PCR (qRT-PCR), 2 µl cDNA, 0.5 µl each reverse primers and forward primer (10 pmol; Macrogen Co.), 10 µl SYBR PremixExTaq II (2 X) (Takara Bio Co.), and 7 µl DNase free water were mixed. In this reaction, *β-actin* was utilized as the control endogenous gene. The primer sequences utilized are represented in Table I. The thermo cycles as follow 95°C for 30 s; 40 cycles of 95°C for 5 s and 60°C for 32 s; 95°C for 15 s, 60°C for 60 s, and 95°C for 1 s. Relative expressions of *HOXC13-AS*, *HOXC13*, *HOTAIR*, *HOXC-AS3*, and *HOXC10* were estimated via the $2^{-\Delta\Delta C_t}$ procedure.

Statistical analysis

Data collected in this study was analyzed by GraphPad Prism version 8. The difference between the two groups was compared through a t-test. Spearman's correlation was utilized for evaluating the association between the relative expression of genes and the clinical parameters.

Results

Expression of selected genes in gastric cancer

The relative expression of *HOTAIR*, *HOXC13*, and *HOXC10* exhibited statistically significant overexpression in tumor samples compared to the normal margin samples. Expression of *HOTAIR*, *HOXC13*, and *HOXC10* was significantly up-regulated up to 17.6 (p value < 0.0001), 12.6 (p value < 0.0001), and 15 (p value < 0.0001) fold, respectively, in the cancer tissue in comparison with those of the marginal tissue. Although the expressions of *HOXC-AS3* and *HOXC13-AS* were upregulated in the tumor tissues compared with the marginal normal tissues with a fold change of 4.9 and 9.46, respectively, statistical analysis showed the difference was not significant (P = 0.9 and p < 0.2772, respectively). (Fig. 1).

Correlation of the expressions of genes in gastric cancer

To explore the association between the expressions of genes in gastric cancer, the fold change ratio of each gene was utilized. From our results, there was a significantly positive correlation between the expressions of *HOXC-AS3* and *HOXC10* (r = 0.52), *HOXC13-AS* and *HOXC13* (r = 0.57), *HOTAIR* and *HOXC-AS3* (r = 0.39), *HOTAIR* and *HOXC13-AS* (r = 0.36), as well as *HOXC13* and *HOTAIR* (r = 0.33) in a moderate correlation (Table 2).

Table 1
Sequence of primers used in this study.

Category	Genes	RNA product	Primer sequences (5'3')
Endogenous control gene	<i>βactin</i>	Coding	F: ATTGGCAATGAGCGGTTC R: TGAAGGTAGTTTCGTGGATG
Target genes	<i>HOXC13-AS</i>	lncRNAs	F: CGGACATCGGAGCACTATG R: CGGCTGGTCTTCTTGAGG
	<i>HOXC-AS3</i>	lncRNAs	F: CGATAGGCGGCTTTGG R: CGTCTTGTGTGCTGGTTTCC
	<i>HOTAIR</i>	lncRNAs	F: GAAAGGTCCTGCTCCGCTTC R: TCCTCTCGCCGCCGTCTG
	<i>HOXC13</i>	Coding	F: TCTCCCTTCCCAGACGTGGT R: CGCTCAGAGAGGTTTCGTGGT
	<i>HOXC10</i>	Coding	F: CCAGACACCTCGGATAACG R: GGCACCTCTTCTTCCTTCC
F, forward; R, reverse.			

Table 2
Correlation between the expressions of genes studied in gastric cancer

	HOXC-AS3 r (p value)	HOXC10 r (p value)	HOTAIR r (p value)	HOXC13-AS r (p value)	HOXC13 r (p value)
HOXC-AS3	-	0.519** (p < 0.003)	0.396* (p < 0.03)	-	-
HOXC10	0.519** (P < 0.003)	-	0.245 ^{NS} (p < 0.193)	-	-
HOXC13-AS	-	-	0.36 * (p < 0.05)	-	0.57** (P < 0.001)
HOXC13	-	-	0.33 ^{NS} (p < 0.07)	0.57** (P < 0.001)	-
*: P < 0.05, **: P < 0.01, ns: not significant.					

Association between gene expression and clinic-pathological properties

HOXC13 and *HOXC10* showed a significant correlation with both distant metastasis and perineural invasion, meaning that the expression of these genes in samples with metastasis and perineural invasion was much higher than samples without this feature. Moreover, the *HOXC13-AS* gene showed a significant relationship with the sex factor, so that in women the expression of this gene was higher than in men (Table 3).

Table 3

Association between the clinic-pathological properties of gastric cancer-suffered peoples and the expression of the studied genes.

	Number (%)	HOXC- AS3 p value	HOXC10 p value	HOTAIR p value	HOXC13- AS p value	HOXC13 p value
Sex						
Male	26 (86.66)	0.52 (NS)	0.39 (NS)	0.54 (NS)	0.03**	0.11 (NS)
Female	4 (13.33)					
Age						
≥ 65	16 (53.33)	0.74 (NS)	0.5 (NS)	0.31 (NS)	0.47 (NS)	0.31 (NS)
> 65	14 (46.66)					
TNM stage						
I	0 (0)	0.06 (NS)	0.16 (NS)	0.78 (NS)	0.09 (NS)	0.51 (NS)
II	1 (3.33)					
III	22 (73.33)					
IV	7 (23.33)					
Tumor size, cm						
≥ 5	19 (63.66)	0.23 (NS)	0.51 (NS)	0.26 (NS)	0.41 (NS)	0.39 (NS)
> 5	11 (36.66)					
Histological grade						
I	5 (16.66)	0.83 (NS)	0.32 (NS)	0.35 (NS)	0.19 (NS)	0.28 (NS)
II	8 (26.66)					
III	16 (53.33)					
IV	1 (3.33)					
Tumor type						
1	23 (76.66)	0.25 (NS)	0.93 (NS)	0.85 (NS)	0.59 (NS)	0.91 (NS)
2	3 (10)					
3	4 (13.33)					

Abbreviation: TNM, Tumor-Node-Metastasis staging system. *: $P < 0.05$, **: $P < 0.01$, ns: not significant.

	Number (%)	HOXC-AS3 p value	HOXC10 p value	HOTAIR p value	HOXC13-AS p value	HOXC13 p value
Lymphatic invasion						
No	7 (23.33)	0.87 (NS)	0.75 (NS)	0.98 (NS)	0.75 (NS)	0.19 (NS)
Yes	20 (66.66)					
N/A	3 (10)					
Perineural invasion						
No	8 (26.66)	0.92 (NS)	0.005*	0.53 (NS)	0.69 (NS)	0.03*
Yes	22 (73.33)					
Metastasis classification						
M0	27 (90)	0.12 (NS)	0.007*	0.35 (NS)	0.02 (NS)	0.29*
M1	3 (10)					
N classification						
N0	1 (3/33)	0.83 (NS)	0.32 (NS)	0.37 (NS)	0.56 (NS)	0.31 (NS)
N1	16 (53/33)					
N2	9 (30)					
N3	4 (13/33)					
Abbreviation: TNM, Tumor-Node-Metastasis staging system. *: P < 0.05, **: P < 0.01, ns: not significant.						

Discussion

The expression of *HOXC* genes is varying across various cancer types. It has been reported that *HOXC* genes were up-regulated in some types of cancers included colorectal (12), gastric (13), and lung (14). Some reports showed their down-regulation in a different type of Larynx squamous cell carcinoma (7). This difference in the expression among various cancers displays that these genes can play either a tumor-suppressive or oncogenic function, depending on the cancer's type (5). According to our findings *HOXD10* was up-regulated in gastric cancer and it was concerned as an oncogenic gene. We showed that *HOXC13* has a significant correlation with both distant metastasis and perineural invasion. Similarly, de Barros e Lima Bueno et al. (7) demonstrated that *HOXD10* leads to enhance cell invasion and migration, inhibition of apoptosis, and decrease of cell survival. Metastasis is a crucial factor influencing gastric tumor prognosis and development. Guo et al. (13) further detected the efficacy of *HOXC10* on gastric tumor metastasis via migration and invasion tests, demonstrating that this gene could promote gastric

tumor metastasis. All together our work exhibited an important function of *HOXC10* in the metastasis and proliferation of gastric tumor. It seems that *HOXC10* regulates this biological progress via MAPK signaling (14), therefore it can provide helpful data for the directed therapy against the gastric tumor.

Recently some reports showed *HOXC13* is up-regulated in lung adenocarcinoma (15), prostate cancer (16), skin tumor (17), and squamous carcinoma (18). In agreement with our findings, Enteghami et al. (12) observed that the *HOXC13* expression was significantly higher in colorectal tumor tissues compared to the normal adjacent tissues. *HOXC13* exhibited a significant correlation with both distant metastasis and perineural invasion in our study. Similarly, Yao et al (15) stated that the expression of this gene was increased significantly in lung adenocarcinoma, and related to poorer prognosis and worse clinical features. It appeared that *HOXC13* enhances progression and cell proliferation through up-regulation of *CCNE1* and *CCND1* (19). Besides, miR-141 was known to be able to impede the cell proliferation of lung adenocarcinoma via repressing the *CCNE1*, *CCND1*, and *HOXC13* expression (20).

We also evaluated the *HOTAIR* expression in gastric tumors and explored its correlation with the clinicopathological properties. Our findings obviously displayed the increased *HOTAIR* expression in tumor tissue relative to non-tumor tissue. This finding in line with Endo et al. (21) who demonstrated up-regulation of the *HOTAIR* gene in gastric tumors in comparison with normal tissues. We did not find any correlation between *HOTAIR* expression and clinic-pathological properties. This is in line with observations of previous reports (22, 23, 24). Emadi-Andani et al. (11) also did not demonstrate a significant correlation between the clinic-pathological properties and the target gene expression. In contrast, Nie et al. (25) found that the *HOTAIR* expression is associated with lymph node metastasis and tumor stage. Moreover, according to our results the expression of *HOXC13* and *HOTAIR* is moderately associated and therefore one of these genes can modulate the expressions of the other, and/or both genes can share regulatory mechanisms of expression. An association between *HOXC13* and *HOTAIR* was also reported in breast and colorectal cancers (12 & 26). Of note, this gene can play a vital role in the progression of gastric tumors.

HOXC-AS3 can regulate a group of genes associated with gastric tumor migration and cell proliferation, such as *HDAC5*, *WNT10B*, and *MMP7* by binding to *YBX1* (4). Among these, *HDAC5* is concerned as a vital gene belonging to the family histone deacetylase (4). Since the histone deacetylation/acetylation process is imperative for gene expression and chromatin remodeling, each imbalance in this process results in abnormal expression of tumor-associated genes (27). In addition to histone deacetylase 5, a couple of genes associated with tumorigenesis are modulated in a similar way by *HOXC-AS3* lncRNA (28). Zhang et al (29) demonstrated that abnormal activity of *HOXC-AS3* can induce cell migration and proliferation in gastric cancer through transcriptional activation of a large number of genes via interactions with *YBX1*. However, the *HOXC-AS3* expression was insignificantly up-regulated in the tumor tissue in comparison to the marginal normal tissue in our study. Similarly, Enteghami et al. (12) showed the expression of *HOXC-AS3* did not differ significantly in colorectal cancer compared to the normal adjacent tissues.

HOXC13-AS has been assumed to involve in the invasion and cell proliferation of nasopharyngeal carcinoma via *miR-383-3p/ HMGA2* axis (30) and to denote a weak prognosis (31). This lncRNA accelerates the malignancy through the *miR-378g/HOXC13* axis, presenting novel thought for facilitating the lncRNA-directed remedy of the cell carcinoma of oral squamous (32). However, the *HOXC13-AS* expression was insignificantly up-regulated in the tumor tissue compared to the marginal tissue in the present study. As a note, the *HOXC13-AS* gene showed a significant relationship with the sex factor, so that in females the expression of this gene was higher than in men.

Conclusion

The current research successfully recognized the differentially expressed genes in gastric cancer. Our findings showed that *HOTAIR*, *HOXC13*, and *HOXC10* were significantly up-regulated in the tumor tissue compared to the margin normal tissue samples (Fig. 1). Also gene expression correlates significantly with clinicopathological characteristics (Table3); finally, we can conclude that *HOTAIR*, *HOXC13*, and *HOXC10* could be considered in gastric cancer diagnosis and therapy. Further studies including bigger sample size and functional analyses could be helpful to gain more accurate data.

Declarations

Ethics approval and consent to participate

The current research was approved by the Ethical Committee of Shahid-Chamran University of Ahvaz, Ahvaz, Iran.

Consent for publication

The informed consent was obtained from all the patients.

Availability of data and materials

The datasets utilized and/or analyzed in this research are available from the corresponding author on request.

Competing interests

Not applicable.

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Authors' contributions

MZ and HG conceived and designed the research; MH and RF acquired, analyzed and interpreted the data; MH and MZ participated in drafting the manuscript. All authors have read and approved the final version of the manuscript.

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References

1. Siegel RL, Miller KD and Jemal A: Cancer Statistics, 2017. *CA Cancer J Clin* 67:7–30, 2017.
2. Sexton RE, Al Hallak MN, Diab M and et al: Gastric cancer: a comprehensive review of current and future treatment strategies. *Cancer Metastasis Rev* 39:1179–1203, 2020.
3. Hu B, Hajj N, Sittler S, Lammert N, Barnes R, and Meloni- Ehrig A: Gastric cancer: classification, histology and application of molecular pathology. *Journal of Gastrointestinal Oncology* 3:251–261, 2012.
4. Tan H, Zhang S, Zhang J, Zhu L, Chen Y, Yang H, Chen Y, An Y, and Liu B: Long non-coding RNAs in gastric cancer: New emerging biological functions and therapeutic implications. *Theranostics* 10:8880-8902, 2020.
5. Pai P and Sukumar S: HOX genes and the NF- κ B pathway: A convergence of developmental biology, inflammation and cancer biology. *BBA – Reviews on Cancer*. <https://doi.org/10.1016/j.bbcan.2020.188450>, 2020.
6. Shah N, Sukumar S: The Hox genes and their roles in oncogenesis. *Nat Rev Cancer* 10(5):361–71, 2010.
7. de Barros e Lima Bueno R, Ramão A, Pinheiro DG, and et al: HOX genes: potential candidates for the progression of laryngeal squamous cell carcinoma. *Tumor Biol* 37:15087–15096, 2016.
8. Ewing C M, Ray AM, Lange EM, Zuhlke KA, Robbins CM, Tembe WD, and et al: Germline Mutations in HOXB13 and Prostate-Cancer Risk. *N Engl J Med* 366:141–149, 2012.
9. Li T, Mo X, Fu L, Xiao B and Guo J: Molecular mechanisms of long noncoding RNAs on gastric cancer. *Oncotarget* 7: 8601-12, 2016.
10. Schmitt AM and Chang HY. Long Noncoding RNAs in Cancer Pathways. *Cancer Cell* 29:452-63, 2016.
11. Emadi-Andani E, Nikpour P, Emadi-Baygi M and Bidmeshkipour A: Association of HOTAIR expression in gastric carcinoma with invasion and distant metastasis. *Adv Biomed Res* 3: 135, 2014.
12. Enteghami M, Ghorbani M, Zamani M, Galehdari H. HOXC10 is significantly overexpressed in colorectal cancer. *Biomedical Reports*. 2020 Sep;13(3):1-.
13. Guo C, Hou J, Ao S, Deng X and Lyu G: HOXC10 up-regulation promotes gastric cancer cell proliferation and metastasis through MAPK pathway. *Chin J Cancer Res* 29(6):572–580, 2017.
14. Tang XL, Ding BX, Hua Y and et al: HOXC10 promotes the metastasis of human lung adenocarcinoma and indicates poor survival outcome. *Front Physiol* 8:557, 2017.

15. Yao Y, Luo J, Sun Q, Xu T, Sun S, Chen M, Lin X, Qian Q, Zhang Y, Cao L, Zhang P and Lin Y: HOXC13 promotes proliferation of lung adenocarcinoma via modulation of CCND1 and CCNE1. *Am J Cancer Res* 7(9):1820–1834, 2017.
16. Komisarof J, McCall M, Newman L, Bshara W, Mohler JL, Morrison C and Land H: A four gene signature predictive of recurrent prostate cancer. *Oncotarget* 8:3430–3440, 2017.
17. Battistella M, Carlson JA, Osio A, Langbein L and Cribier B: Skin tumors with matrical differentiation: lessons from hair keratins, betacatenin and PHLDA-1 expression. *J Cutan Pathol* 41:427–436, 2014.
18. Marcinkiewicz KM and Gudas LJ: Altered histone mark deposition and DNA methylation at homeobox genes in human oral squamous cell carcinoma. *J Cell Physiol* 229:1405–1416, 2014.
19. Li C, Cui J, Zou L, Zhu L and Wei W: Bioinformatics analysis of the expression of HOXC13 and its role in the prognosis of breast cancer. *Oncol Lett* 19: 899-907, 2019.
20. Kasiri S, Ansari KI, Hussain I, Bhan A and Mandal SS: Antisense oligonucleotide mediated knockdown of HOXC13 affects cell growth and induces apoptosis in tumor cells and over expression of HOXC13 induces 3D-colony formation. *RSC Adv* 3:3260–3269, 2013.
21. Endo H, Shiroki T, Nakagawa T, Yokoyama M and et al: Enhanced expression of long non-coding RNA HOTAIR is associated with the development of gastric cancer. *PLoS One* 8(10):e77070, 2013.
22. Nakagawa T, Endo H, Yokoyama M, Abe J, Tamai K et al. (2013) Large noncoding RNA HOTAIR enhances aggressive biological behavior and is associated with short disease-free survival in human non-small cell lung cancer. *Biochem Biophys Res Commun* 436: 319-324.
23. Hajjari M, Behmanesh M, Sadeghizadeh M and Zeinoddini M: Upregulation of HOTAIR long non-coding RNA in human gastric adenocarcinoma tissues. *Med Oncol* 30: 670, 2013.
24. Yang Z, Zhou L, Wu LM, Lai MC, Xie HY and et al: Overexpression of long noncoding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation. *Ann Surg Oncol* 18:1243-1250, 2011.
25. Nie Y, Liu X, Qu S, Song E, Zou H and Gong C: Long non-coding RNA HOTAIR is an independent prognostic marker for nasopharyngeal carcinoma progression and survival. *Cancer Sci* 104:458–6, 2013.
26. Kogo R, Shimamura T, Mimori K, Kawahara K, Imoto S and et al: Long noncoding RNA HOTAIR regulates polycomb-dependent chromatin modification and is associated with poor prognosis in colorectal cancers. *Cancer Res* 71:6320–6326, 2011.
27. Su J, Yu B, Zhang C, Yi P, Li H, Xu C, Cao L, Chen P, Li M, Shen K and Chen J: Long noncoding RNA HOXC-AS3 indicates a poor prognosis and regulates tumorigenesis by binding to YBX1 in breast cancer. *Am J Transl Res* 12(10):6335-6350, 2020.
28. Yang Z and Hu T. Long noncoding RNA HOXC-AS3 facilitates the progression of invasive mucinous adenocarcinomas of the lung via modulating FUS/FOXM1. *In Vitro Cell Dev Biol Anim* 56:15-23, 2020.
29. Zhang E, He X, Zhang C, and et al: A novel long noncoding RNA HOXC-AS3 mediates tumorigenesis of gastric cancer by binding to YBX1. *Genome Biol* 19:154, 2018.

30. Gao C, Lu W, Lou W, Wang L and Xu Q: Long noncoding RNA HOXC13-AS positively affects cell proliferation and invasion in nasopharyngeal carcinoma via modulating miR-383-3p/HMGA2 axis. *J Cell Physiol* 234:12809–20, 2019.
31. Li X, Wang Q, Rui Y, Zhang C, Wang W, Gu J, Tang J and Ding Y: HOXC13-AS promotes breast cancer cell growth through regulating miR-497-5p/PTEN axis. *J Cell Physiol* 234(12):22343-22351, 2019.
32. Li W, Zhu Q, Zhang S, Liu L, Zhang H, Zhu D: HOXC13-AS accelerates cell proliferation and migration in oral squamous cell carcinoma via miR-378g/HOXC13 axis. *Oral Oncol* 111:104946, 2020.

Figures

Figure 1

The expression of HOXC13-AS, HOXC13, HOTAIR, HOXC-AS3, and HOXC10 in the gastric cancer tissue. Only the expression levels of HOTAIR, HOXC13, and HOXC10 were significantly different between the two tissues. **: $P < 0.01$, ****: $P < 0.0001$, ns: not significant.