Life Sciences 217 (2019) 271–282

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie

Long non-coding RNA Hotair promotes gastric cancer progression *via* miR-217-GPC5 axis

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ARTICLE INFO

Keywords:
Hotair
Epithelial mesenchymal transition
microRNA-217
Gastric cancer
GCP5

ABSTRACT

Aims: The oncogenic role of lncRNA Hotair has been acknowledged in subset of malignancies, including gastric cancer (GC). However, the detailed molecular mechanisms that contribute to its oncogenic role of are largely elusive. This study was designed to explore the underlying mechanism that contributes the regulatory role of Hotair in GC pathogenesis and progression.

Main methods: Expression pattern of lncRNAs in GC tissues and adjacent normal tissues were identified by using microarray analysis. The cell proliferation of GC cells was examined by CCK-8 assay and colony formation assay, while migration and invasion capabilities of GC cells were examined by Transwell (with or without Matrigel) assay. Cell apoptosis was examined by Flow cytometer. qRT-PCR and western blotting were used to examine the expression of Hotair, miR-217, and other related genes. The potential target relationships were predicted by miRcode algorithm, and validated by dual luciferase reporter gene assay.

Key findings: We observed that Hotair was frequently up-regulated in GC tissues and cell lines, and high Hotair level was positively correlated with poor prognosis in GC patients. Knockdown of Hotair inhibited GC cells' viability, migration, invasion, Epithelial mesenchymal transition process. MiR-217 was decreased while GCP5 was increased in GC cells. Hotair negatively regulated the expression of miR-217 in GC while miR-217 targeted GCP5 to down-regulate its expression. Hotair promoted GC development by promoting GCP5 expression *via* sponging miR-217.

Significance: Hotair could serve as a potentially prognostic indicator and provide new light into its underlying biological-molecular mechanism in GC.

1. Introduction

Gastric cancer (GC) is the second leading cause of cancer mortality in both men and women worldwide [1]. There are approximately 679,100 new GC cases and 498,000 deaths in 2012 in China [2]. The most common risk factors for GC include dietary and environmental factors, H.pylori infection, genetic factor, smoking and precancerous disease [3]. Conventional treatment opinions for GC patients include endoscopic treatment, surgical treatment, chemotherapy, chemotherapy [4]. Although great strides in the diagnosis and treatment of GC has been achieved in recent decades, the overall survival rate for moderate advanced GC patients remains unsatisfactory [5]. Surprisingly, according to the temporal trend analyses, the age-standardized incidence and mortality ratio of GC have an obvious downward trend in recent years [2].

Long non-coding RNAs (LncRNAs) are a novel class of non-coding RNAs that has implicated in a variety of pathological progresses [6]. The available literature indicates lncRNAs contributes to tumorigenesis and progression mainly through regulating multiple cellular processes, such as cell growth, invasion, metastasis, EMT, and posttranscriptional processing [7,8]. EMT plays a crucial role in the initiation of distant metastasis [9,10]. Therefore, targeting EMT represents a promising option for GC prevention and treatment. Wu *et al* reported that lncRNA CASC15 enhanced GC migratory and invasive capacities through

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https://doi.org/10.1016/j.lfs.2018.12.024
Received 3 November 2018; Received in revised form 5 December 2018; Accepted 13 December 2018
Available online 14 December 2018

promoting EMT process [11]. Similarly, Yan et al. found lncRNA SNHG6 exerted oncogenic role through epigenetically silencing p27 and sponging miR-101-3p, and subsequently regulate GC cell growth and EMT progress [12]. In contrast, LncRNA RP11-789C1.1 was identified to repress GC EMT *via* modulating miR-5003/E-cadherin axis.

Hotair is an lncRNA in the mammalian HOXC locus [13]. Recently, many studies reported that high HOTAIR level has been identified as a potential prognostic predictor for tumorigenesis and progression. This notion has been validated in a various types of cancers, including hepatocellular carcinoma [14], renal cell carcinoma [15], breast cancer [16], esophageal cancer [17]. However, to date, little studies have been reported to investigate the functional role of Hotair on GC.

lncRNA might function as a ceRNAs through sponging target miRNAs and indirectly modulate miRNA target genes level [18]. A plethora of studies have reported that various lncRNAs modulate EMT process through sequestrating target miRNAs and indirectly regulated the expression level of critical EMT mediators, including Snail, Twist, E-cadherin, and N-cadherin [19]. For example, LncRNA linc00673 regulated EMT progress through direct sponging miR-150-5p and increasing ZEB1 level in NSCLC [20]. This is consistent with the observation by Li *et al* that lncRNA HULC promoted EMT *via* acting as a ceRNA of miR-200a-3p and subsequently affected the expression pattern of ZEB1 in HCC [21]. As reported in previous study, overexpression miR-217 inhibited GC cell proliferation, migration, and EMT through directly targeting Glypican-5 [22]. Combined preliminary results and bioinformatics prediction, we investigate whether Hotair regulated GC growth and metastasis through acting as ceRNA.

Despite several lines experimental studies has increased our understanding of the cell-biological and molecular mechanisms of Hotair in disease, including cancer. How Hotair regulates biological behavior of tumor cells has so far not been fully elucidated. In current study, we found that Hotair expression was aberrantly up-regulated in GC tissues and cell lines, both *in vivo* and *in vitro* experiments confirmed that Hotair was able to promote GC growth and metastasis.

2. Materials and methods

2.1. Cell lines

A human normal gastric epithelial cell line (GES-1), two human gastric cancer cell lines (SGC-7901, BGC-823) were purchased from Cell Bank of Chinese Academy of Sciences (Shanghai, China). SGC-7901 and BGC-823 were maintained in RPMI 1640 medium (Gibco) and GES-1 was cultured in DMEM (Gibco) medium contained with 10% FBS and 100 U/mL streptomycin and penicillin. Then cells were cultured in a humidified atmosphere at 37 °C and 5% CO_2.

2.2. Tissue sample

32 pairs human GC tissue specimens and matching adjacent normal gastric tissues were taken from GC patients who undergone surgery procedure at the Affiliated Hospital of Kunming Medical University. Fresh samples were sampled and stored at −80 °C. Histological examination was performed by 2 independent senior oncologists using H& E staining to confirm diagnosis. All patients have not accepted any radiotherapy or chemotherapy before operation. Prior to undertaking this experiment, informed consent and ethical clearance were obtained from patients and ethics committee of The Affiliated YanAn Hospital of Kunming Medical University, respectively.

2.3. Microarray profiling

Total RNA was extracted from 5 human GC tissues and corresponding adjacent non-tumor tissues using TRIzol Reagent (Invitrogen, Carlsbad, CA) and purified with a RNeasy Mini Kit (Qiagen, Valencia, CA). The lncRNA microarray analysis was performed as described in previous study [23].

Table 1
Primers for qRT-PCR.

Gene	Sequence
Hotair forward	5′-GCGCTGCAAGTGCTTACTGTGCCA-3′
Hotair reverse	5′-CCGAGGTATTCGCACTGGATAT-3′
miR-217 forward	5′-GGTCTACAAAGGGAAGC-3′
miR-217 reverse	5′-TTTGGCACTAGCACATT-3′
GPC5 forward	5′-A GACCACCACAAGGAACAGTG-3′
GPC5 reverse	5′-AGACTGGGCTTTGA TTCCATT-3′
U6 forward	5′-ATCCTTACGCACCCAGTCCA-3′
U6 reverse	5′-GAACGCTTCACGAATTTGC-3′
GAPDH forward	5′-TGGTCACCAGGGCTGCTT-3′
GADPH reverse	5′-AGCTTCCCGTTCTCAGCC-3′

Forward: Forward primer; Reverse: Reverse primer.

2.4. Quantitative real-time PCR (qRT-PCR) analysis

Total RNA in frozen specimens or cultured GC cells was extracted using Trizol (Invitrogen) following manufacturer's protocols. The relative level of LncHotair, miR-217, and GPC5 were examined by qRT-PCR and calculated using $2^{-\Delta\Delta CT}$ method according to the protocols described in previous study [22]. The primer's sequences for Hotair, miR-217, GPC5, U6, and GADPH were designed and synthesized by Shanghai Genechem company and were shown in Table 1. The expression levels of LncRNA Hotair and miR-217 were normalized to U6, while GCP5 level was normalized to GADPH.

2.5. Cell transfection

To generate HOTAIR stable overexpression vector, HOTAIR cDNA was cloned into pcDNA3.1 vector (Invitrogen, Carlsbad, CA, USA). MiR-217 mimic, negative control oligonucleotides (miR-NC), small interfering RNA targeting HOTAIR (siHOTAIR), scramble siRNA of HOTAIR (siRNA control) were synthesized by RiboBio (Shanghai, China). The GC cells were cultured in 6-well plates and transfected plasmid or inhibitor using Lipofectamine 2000 (Invitrogen, USA). Following transfection 48 h, cells were harvested and used for further experiments.

2.6. CCK-8 assay

Cultured SGC-7901, BGC-823 cells infected with Hotair vector, empty vector, Hotair siRNA, Control siRNA, miR-217 mimic, or control were seeded in 96-well plates at the concentration of 5×10^3/well. Following incubation for 24 h, 48 h, and 72 h, CCK-8 solution was added to each well and incubated in 37 °C for 2.5 h. Then the absorbance was analyzed at 450 nm using a microplate reader (Sunrise). Data represent three independently repeated experiments.

2.7. Colony formation assay

Colony formation assay was performed to evaluate cell proliferative ability. In brief, after SGC-7901, BGC-823 cells transfection with Hotair vector, empty vector, Hotair siRNA, siRNA control, and miR-217 mimic, cells were plated into 6 well plate at the density of 500 cells/well. Following culture for 14 days, cells colonies were fixed with 4% formaldehyde solution for 10 min and then stained with 0.2% crystal violet. Cell images were then visualized and photographed under an inverted microscope and the colonies were calculated using image J (National Institutes of Health, USA).

2.8. Cell apoptosis assays

Apoptotic cell ratio was quantified by flow cytometry following incubation with Annexin V-FITC/PI kit (BD Pharmingen, NJ, USA). In

brief, after GC cells transfected with corresponding overexpression vector or siRNA for indicated time, cell were harvested at the concentration of 1×10^5, and incubation with Annexin V-FITC/PI in dark for 15 min, and detected apoptotic cells using flow cytometry (FACScan, BD Biosciences, USA).

2.9. Transwell migration and invasion assay

The migration and invasion capacities of GC cells were examined using Transwell (8.0 μm/pore, Corning, USA) coated without or with Matrigel, respectively. Briefly, 1×10^5 SGC-7901 or BGC-823 cells transfected with pcDNA3.1-Hotair, Hotair siRNA, empty, siRNA control, and miR-217 mimic in 100 μM medium were added to the upper chamber. Following treatment for indicated time, the remaining cells on the upper chamber were scraped using cotton swabs. Then cells were fixed with 4% formaldehyde solution for 15 min and stained using 0.2% crystal violet for 5 min. Migrated and invasive cells in five random fields were visualized and captured under an inverted microscope (200 × magnification).

2.10. Western blot analysis

Western blotting analysis was performed according to the procedures described in previous study [24]. The antibodies used in this study were listed as follows: rabbit polyclonal Cleaved caspase-3 (no.2302. 1 μg/mL), cleaved PARP (no.32064. 1:1000), E-cadherin (no.2302. 1:1000), N-cadherin (no.2302. 1:1000), Vimentin (no.71144. 1:5000), GCP5 (no.168325. 1:1000), β-catenin (no.16051. 0.25 μg/ml), and Ki-67 (no.92742. 1:2000) antibodies were purchased from Abcam (Cambridge, MA, USA). Anti-β-actin (no.3873. 1:1000), anti-IgG-HRP or anti-mouse IgG-HRP (no.1921, 1:200) were obtained from Bosterbio (China).

2.11. Immunofluorescence staining

Cells were seeded at cover slides, then transfection cells with Hotair vector, empty vector, Hotair siRNA, siRNA control, and miR-217 mimic for 48 h. Cells crawled on slide were harvested and examined E-cadherin and N-cadherin by using immunofluorescence staining. The cells were fixed with 4% formaldehyde solution for 15 min, permeabilized with 0.1% Triton™ X-100 for 10 min, blocked with 5% BSA for 40 min. Rabbit polyclonal against E-cadherin (Sigma, 1:200) and N-cadherin (Sigma, 1:100) were incubated with cells at 4 °C for the night. After washing three time using PBS, incubation cell with Donkey anti-Rabbit and Donkey anti-Mouse IgG (H + L) Highly Cross-Adsorbed Secondary Antibody for 1 h at room temperature, and then counterstained with DAPI (1 μg/mL, 5 min, RT). Cells were observed and photographed under a fluorescence microscope (Olympus, Tokyo, Japan).

2.12. Luciferase reporter assay

5×10^4 GC cells (SGC-7901and BGC-823) were plated in 24 well plates and cultured for overnight. Then pGL3-Hotari WT, pGL3-Hotari–MUT, miR-217 mimic and mimic NC were co-transfected into cells, respectively. Following transfection for 24 h, luciferase activity levels were determined using the dual-luciferase reporter Assay Kit (Promega).

2.13. Xenograft model of gastric cancer

5-week-old male BALB/c-nude mice were randomly allocated into two groups ($n = 6$), SGC7901 xenograft model was established by injecting 1×10^7 Hotair vector or empty vector transfected cells into the left flanks of mice. Mice body weight and tumor volume were measured twice a week and estimated volume according the formula ($V = 1/2 \times D \times d^2$). Upon injection for 24 days, tumors were dissected from mice and stored at −80 °C for the following experiments.

2.14. Statistical analysis

Results are expressed as Mean ± s.d of three independently repeated experiments. Comparisons between two groups were analyzed using one-way analysis of variance followed by Dunnett *t*-test. Correlation between Hotair expression and clinical features were analyzed using Spearman's rank correlation. Survival analysis was calculated using Kaplan-Meier curve. Statistical analyses were conducted by using SPSS 19.0 software or GraphPad Prism 7.0 software. A P value < 0.05 was considered to be statistically significant.

3. Results

3.1. LncRNA Hotair is frequently overexpressed in gastric cancer tissues and cell lines

There is accumulating evidence from both clinical and experimental examinations that lncRNAs is closely implicated in the development and progression of GC. To better understand which lncRNA contributes to GC carcinogenesis, lncRNA microarray analysis was applied to screen differentially expressed lncRNAs between GC tissues and corresponding adjacent non-tumor tissues from 5 pair patients. As illustrated in Fig. 1A, among these abnormally expressed lncRNAs, lncRNA Hotair exhibits the highest expression level in GC tissues samples as compared with their matching adjacent normal tissues. Thus we chose Hotair as our target to investigate its function role and underlying mechanism in GC. Initially, we further verified the results from microassay analysis in a relatively large sample ($n = 32$) GC tissues and adjacent non-tumor tissues using qRT-qPCR. Consistent with preliminary results, as depicted in Fig. 1C, results reveals that Hotair was aberrantly up-regulated in GC specimens, in comparison with control. We also examined Hotair level in cultured GC cell lines, SGC7901 and BGC823. As anticipated, Hotair levels were significantly higher in SGC7901 and BGC823 than in GES-1(normal gastric epithelial cells) (Fig. 1E). Collectively, these data reveal that the abnormal expression of Hotair might play a pivotal role in the development of GC.

3.2. The high levels of Hotair is tightly related to clinicopathological features and poor prognosis in patients with GC

To identify the clinical significance of high Hotair level in GC patients, we assessed the correlation between Hotair expression level and GC patient's clinicopathological features, such as age, gender, tumor size, differentiation status, lymph node invasion, distant metastasis, peritoneal dissemination, TNM stage, survival time. As illustrated in Fig. 1C and Fig. 1D, high Hotair levels were notably related to tumor metastasis and TNM stage in GC patients. Additionally, Kaplan-Meier analysis results demonstrated high Hotair expression levels was tightly associated with poor outcomes in GC patients. GC patients with low Hotair level tended to have a longer survival time, in comparison with High Hotair levels GC patients (Fig. 1F). Indicating Hotair could serve as an independent indicator for poor prognosis. What's moreover, as summarized in Table 2, we found that high Hotair level was also closely related to tumor size ($P = 0.0216$), lymph node metastasis ($P = 0.0145$) and advanced TNM stage ($P = 0.0187$). Nevertheless, no obvious correlation between Hotair level and other clinical features was found. In summary, these results suggest Hotair was closely associated with the carcinogenesis, progression and survival of patients with GC.

3.3. Hotail overexpression reinforces GC cell growth, migration, invasion, EMT and avoids apoptosis

To address the biological functions of Hotair in GC, SGC7901 and BGC823, two well-established GC cell lines, were chosen to conduct

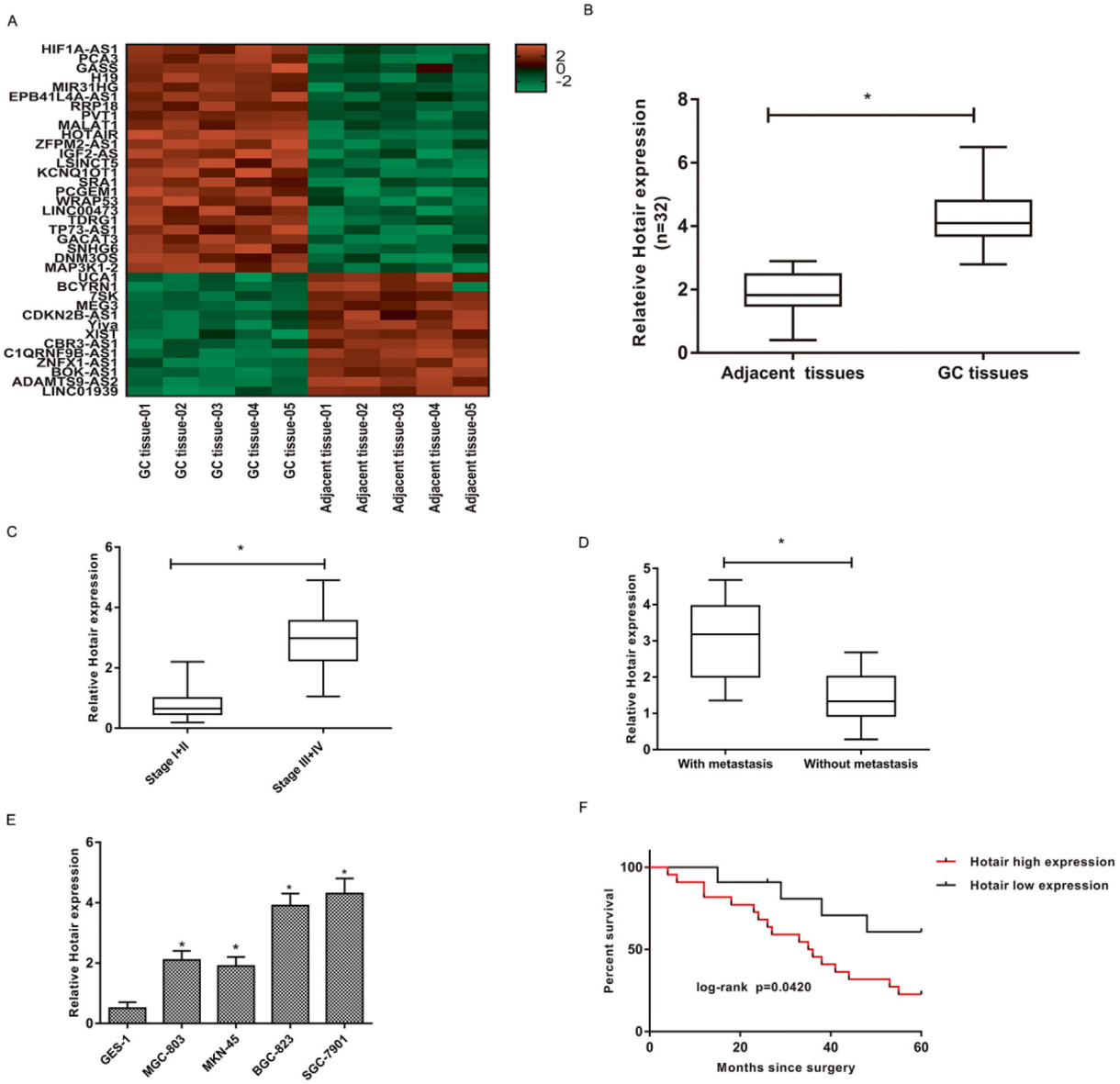

Fig. 1. Differentially expressed of Hotair in GC tissues and cell lines. A. The differentially expressed of lncRNAs between GC tissues and matching adjacent normal tissues were detected using lncRNA microarray analysis. B. Expression level of Hotair was examined in GC tissues and adjacent normal tissues using qRT-PCR. C. qRT-PCR analysis Hotair level in two GC cell lines (GSC7901 and BGC-823) and one normal gastric epithelial cell line (GES1). D. qRT-PCR of Hotair expression levels in patients with (n = 25) or without distant metastasis (n = 25). E. The relative expression of Hotair in normal gastric epithelial cell line GES-1 and GC cell lines (MGC-803, MKN-45, BGC-823, SGC-7901), as determined by qRT-PCR. F. Kaplan-Meier curve for survival time of GC patients with high (n = 20) and low (n = 20) levels of Hotair.

loss-of-function and gain-of-function experiments. We first constructed Hotair overexpression vector using pcDNA3.1, and transfected pcDNA3.1-Hotair or empty vector into SGC7901 and BGC823 cells. Following transfection 48 h, Hotair level in GC cells was validated by using qRT-PCR. As presented in Fig. 2A, Hotair expression level was remarkably increased after transfection pcDNA3.1-Hotair in SGC7901 and BGC823 cells, compared with control. Influence of Hotair on the proliferation abilities of SGC7901 and BGC823 cells was evaluated using CCK-8 assay. Cell viability analysis revealed that overexpression Hotair significantly enhanced the proliferative ability of SGC7901 and BGC823 cells (Fig. 2B–C). The promoting effect of Hotair on cell proliferation was further confirmed using colony formation assay. Likewise, results confirmed that Hotair overexpression effectively enhanced cell colony formation ability (Fig. 2D), as evidenced by the increase number of colonies in SGC7901 and BGC823 cells transfected with pcDNA3.1-Hotair.

To investigate whether the effect of Hotair overexpression on cell growth was related to apoptosis, we measured apoptotic cell ratio in SGC7901 and BGC823 cells using flow cytometer analysis. Fig. 2E shows that Hotair vector-transfected SGC7901 and BGC823 cells had a relatively lower percentage of apoptotic cells as compared with empty control. Indicating Hotair overexpression effectively protected SGC7901 and BGC823 cells from apoptotic cell death. There is compelling evidence demonstrated that lncRNAs involved tumorigenesis and progression through modulating EMT progress, which subsequently enhanced the migratory and invasive properties of tumor cells, and led to the occurrence of distant metastasis [23]. More recently, a plethora of research has reported the positive correlation between Hotair expression level and EMT progress in various tumor types [25,26]. Therefore, we furthermore examined the influences of Hotair on GC cell migration, invasion and EMT progression. We first examined the effect of Hotair on the migratory and invasive properties of SGC7901 and

Table 2
Correlations between Hotair expression and clinicopathological characteristics in gastric cancer patients.

Characteristics	Hotair expression		*P* value
	High (22)	Low (10)	
Age			0.1667
≤50	14	3	
> 50	8	7	
Sex			0.5878
Male	9	6	
Female	13	4	
Tumor size			0.0216*
≤5 cm	16	4	
> 5 cm	6	6	
Histological grade			0.6111
Well, moderate	13	7	
Poor	9	3	
TNM stage			0.0187*
I and II	3	4	
III and IV	19	6	
Distant metastasis			0.0385*
Absent	18	8	
Present	4	2	
Lymph node metastasis			0.0145**
Absence	12	6	
Presence	10	4	

* $P < 0.05$.

BGC823 cells, the findings demonstrated Hotair overexpression notably enhanced the migration and invasion capacities of GC cells (Fig. 2F–G). In view of the importance of EMT on cell metastasis, we subsequently examined the protein levels of EMT marker genes, including epithelial marker genes (E-cadherin), and mesenchymal marker genes (N-cadherin, vimentin, and β-catenin), in SGC7901 and BGC823 cells transfected with Hotair vector or empty vector. Similarly, we observed that Hotair overexpression, compared with empty vector transfected cells, could significantly down-regulate E-cadherin expression and simultaneously increase N-cadherin, Vimentin, and β-catenin levels in SGC7901 and BGC823 cells (Fig. 2H–I). Afterwards, immunofluorescence staining results also supported that Hotair overexpression significantly promotes EMT progress in GC cells, as evidenced by the decrease of E-cadherin positive cells and increase in N-cadherin positive cells. Taken together, these results suggest Hotair could serve as an oncogenic lncRNA in GC.

3.4. Down-regulation Hotair reduces GC cell growth, migration, metastasis, EMT and enhances apoptotic cell death in vitro

To further validate the oncogenic roles of Hotair in GC, we knocked down Hotair expression in SGC7901 and BGC823 by specific siRNAs transfection. Hotair level was effectively decreased in Hotair siRNA transfected GC cells as determined by qRT-PCR (Fig. 3A). As shown in Fig. 3B–D, we found that knockdown of Hotair level effectively inhibited GC cell proliferation and reduced the capacity of colony formation as compared with siRNA control transfected cells. Flow cytometric analysis demonstrated that down-regulation of Hotair notably promoted apoptotic cell death (Fig. 3E). Furthermore, Transwell assays indicated that knockdown of Hotair level significantly decreased cell migratory and invasive abilities (Fig. 3F–G). Based on the results of western blotting analysis and immunofluorescence staining, we also confirmed that knockdown of Hotair could significantly increase E-cadherin expression and decreased expression of N-cadherin, vimentin and β-catenin (Fig. 3H–J). Collectively, these data further strengthen Hotair serves as an oncogene in the tumorgenesis and progression of GC.

3.5. Hotair promotes tumor growth and metastasis in vivo

To further confirm the oncogenic roles of Hotair *in vivo*, we first examined whether Hotair affected tumor growth using a subcutaneous xenograft tumor model. As depicted in Fig. 4A and D, the tumor volume was obviously larger in mice injecting Hotair vector transfected SGC7901 cell as compared with empty vector transfected cells. Similarly, the average of tumor weight in Hotair-overexpressing nude mice was significantly larger than those in vector control (Fig. 4B). Afterwards, we examined the proteins level of several markers, including Ki-67, Cleaved caspase-3, E-cadherin, N-cadherin, Vimentin, and β-catenin, which were used to indicate the effect of Hotair on cell proliferation, apoptosis and EMT progress *in vivo*. Results demonstrated that overexpression Hotair caused an increase in Ki-67 level, while decrease the cleavage of caspase-3 (Fig. 4C). Indicating Hotair promoted GC tumor cell proliferation and evading apoptosis. Consistent with *in vitro* results, overexpression Hotair increased EMT phenotype in tumor tissue, as supported by the decrease E-cadherin level and increase of N-cadherin, Vimentin, and β-catenin (Fig. 4C). In summary, these results suggest that Hotair level tightly associated with *in vivo* growth and metastasis capacities of GC cells. In summary, the findings further support the idea that Hotair was involved in the growth and metastasis of GC.

3.6. Hotair functioned as a ceRNA by sponging miR-217 and indirectly up-regulated GCP-5 expression in GC

In order to clarify the underlying mechanism which contributes to the oncogenic function roles of Hotari, computer algorithm miRanda was applied to predict the potential miRNA targets of Hotair in GC. In total, 15 miRNAs were found to have the binding site for Hotair (miR-217, miR-138 and miR-23b-3p), among these miRNAs, miR-217, which has been reported to function as a tumor suppressor through targeting GCP5 in GC, attracted our attention. Thus, miR-217 was chosen as Hotair potential target for the deep research. The putative binding sites in miR-217 for Hotair were depicted in Fig. 5A. A dual luciferase reporter assay was applied to further confirm our prediction that miR-217 was a potential target for Hotair in GC cells. As shown in Fig. 5B and C, overexpression of miR-217 effectively reduced the luciferase activity of psi Hotair WT, not psi Hotair WT MUT. Suggesting that miR-217 could directly targeted by Hotair in GC. To further validate the surmise that Hotair promotes GC cells growth and metastasis through directly targeting miR-217. Next, we detected the expression level of miR-217 in GC tissues and cell lines using qTR-PCR, and then analyzed the correlation between Hotair level and miR-217 expression using Spearman's correlation analysis. Our previous studies revealed that miR-217 expression was notably lower in GC tissues than correspondingly adjacent normal tissues. Similarly, miR-217 was also notably down-regulated in GC cell lines, SGC7901 and BGC823, than the normal gastric epithelial cell lines, GES-1 [22]. Based on preliminary findings, we hypothesized that the interaction action between Hotair and miR-217 might co-contribute to the development of GC·As expected, spearman's correlation analysis indicates that miR-217 level was negatively related to Hotair expression in GC cells (Fig. 5E). In addition, the level of miR-217 in Hotair siRNA, siRNA control, pcDNA3.1-Hotair or empty vector transfected SGC7901 and BGC823 cells was examined by qTR-PCR. Consistent with above results, results demonstrated that miR-217 level reduced significantly after transfection of pcDNA3.1-Hotair in SGC7901 and BGC823 cells, while down-regulation dramatically increased miR-217 level (Fig. 5D). Furthermore, an early study has proved that miR-217 exerts tumor suppressor function through directly targeting oncogene, Glypican-5 (GPC5) in GC [22]. Accordingly, we also determined the mRNA and protein levels of GPC5 in SGC7901 and BGC823 cells transfected with Hotair siRNA, siRNA control, pcDNA3.1-Hotair or empty vector. Fig. 5F and C indicates that GPC5 abundance was significantly increased at both mRNA and protein levels in SGC7901 and

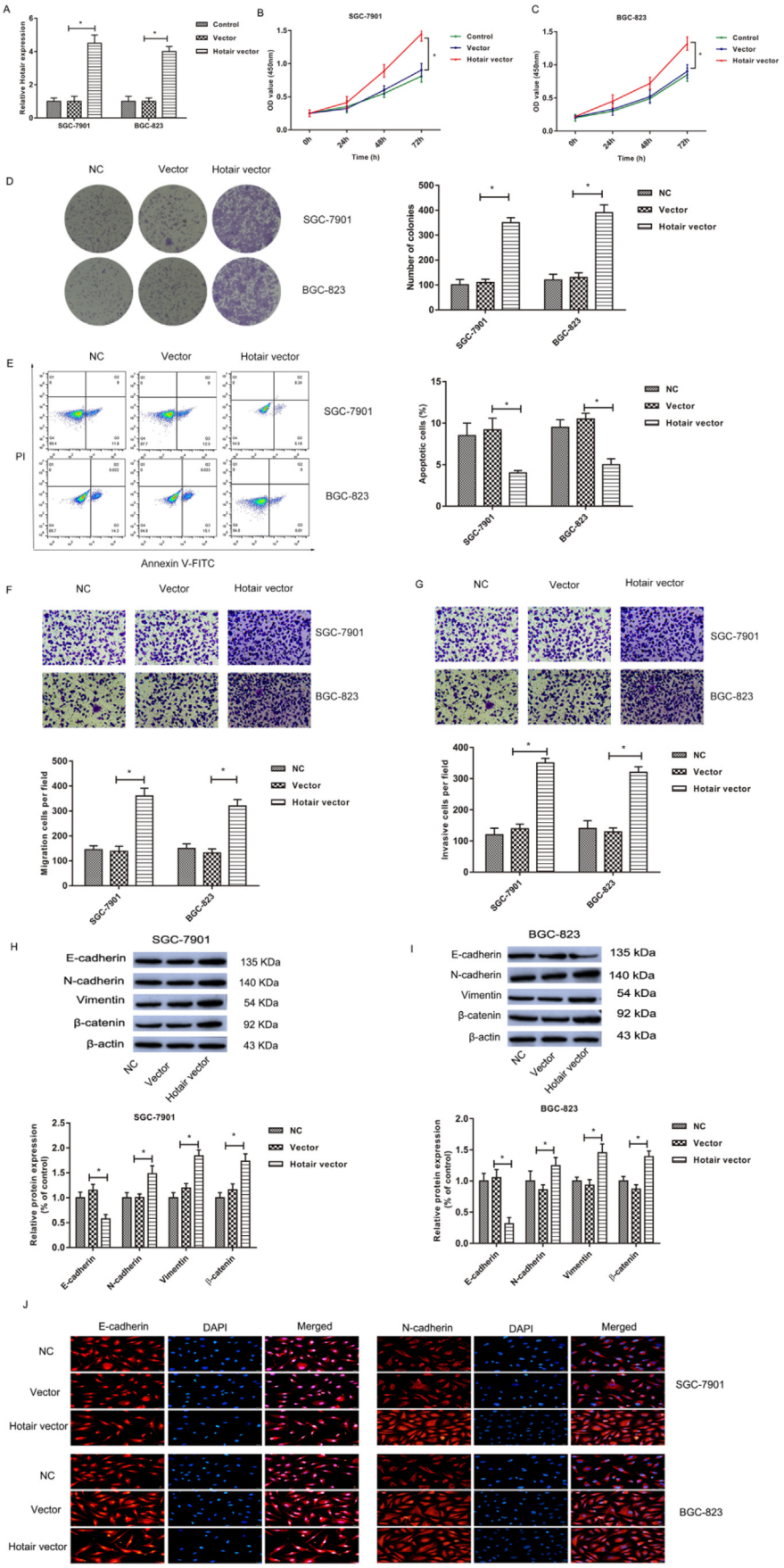

Fig. 2. Overexpression of Hotair promotes GC cell proliferation, colony formation, migration, invasion, EMT and avoids cell apoptosis. A. Following GSC7901 and BGC-823 cells transfected with pcDNA3.1-Hotair vector or empty vector, overexpression of Hotair were confirmed by qRT-PCR. B. Effect of Hotair overexpression on the cell proliferation of GSC7901 and BGC-823 cells was examined using CCK-8 assay. C. Colony formation assay was used to further confirm the promoting effect of Hotair on GC cell proliferation. D. Flow cytometric analysis the influence of Hotair on GSC7901 and BGC-823 cells apoptosis. F,G. Transwell assays were performed to evaluate the effect of Hotair on cell migration and invasion abilities in GSC7901 and BGC-823 cells. G. Western blotting analysis the expression level of E-cadherin, N-cadherin, Vimentin, β-catenin in Hotair vector or empty vector transfected GSC7901 and BGC-823 cells. H. Immunofluorescence staining of E-cadherin and N-cahderin expression in Hotair vector or empty vector transfected GSC7901 and BGC-823 cells.

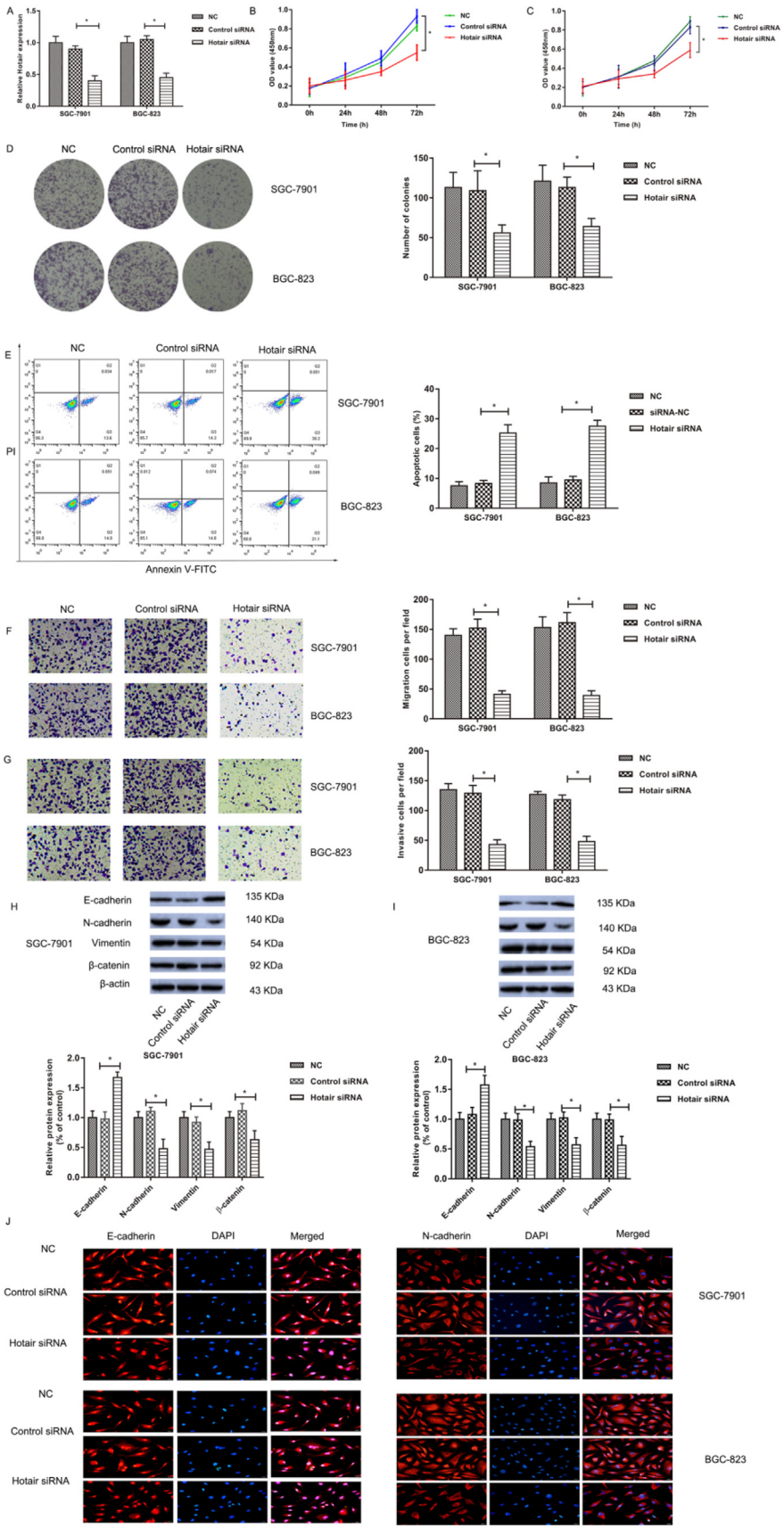

Fig. 3. Effect of Hotair silencing on GC cell proliferation, apoptosis, migration, invasion and EMT process. A. Knockdown Hotair expression using siRNA was verified by qRT-PCR in GSC7901 and BGC-823 cells. B. CCK-8 assay and colony formation assay were applied to examine the effect of Hotair knockdown on cell growth and proliferation. D. The morphology of GSC7901 and BGC-823 cells transfected with Hotair siRNA or control siRNA were shown. E. Flow cytometric analysis the influence of Hotair knockdown on GSC7901 and BGC-823 cells apoptosis. E, F. The effect of Hotair knockdown on cell migration and invasion was assessed by Transwell assay. G. The expression of EMT marker genes, E-cadherin, N-cadherin, Vimentin, β-catenin were detected in Hotair deletion GSC7901 and BGC-823, as determined by western blotting. H. Immunofluorescence staining of E-cadherin and N-cadherin expression in control siRNA or Hotair siRNA transfected GSC7901 and BGC-823 cells.

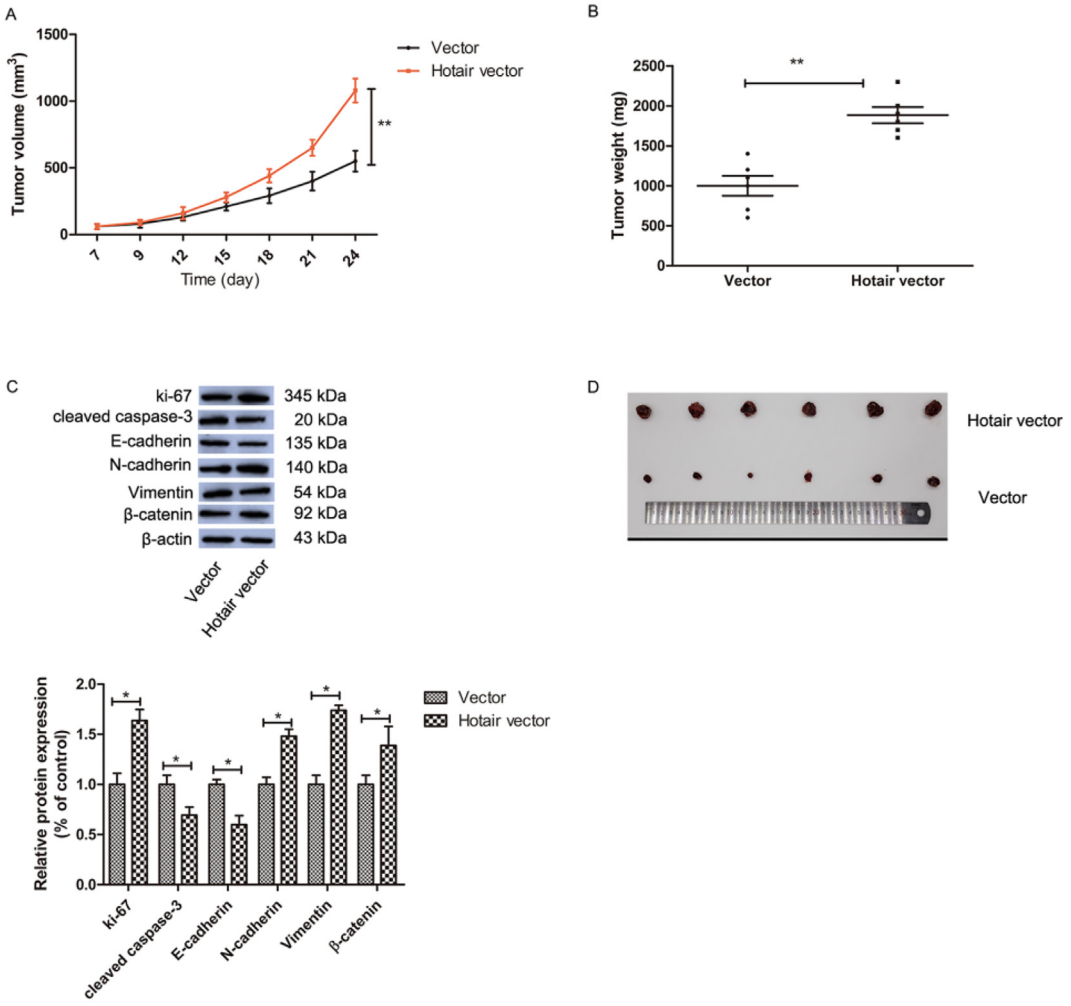

Fig. 4. Overexpression of Hotair enhanced GC growth and metastasis in nude mice. A. Tumor volume were measured twice a week and estimated according the formula ($V = 1/2 \times D \times d^2$). B. Tumor weight was weighed after tumor dissection from nude. C. Representative picture of surgically resected tumors from BALB/c-nude. D. Western blotting analysis of Ki67, cleaved caspase-3, N-cadherin, E-cadherin, vimentin, β-catenin proteins in the xenografts tumor. E.F. Counting the number of metastatic nodules in livers and lungs in nude mices transfected with Hotair vector or empty vector cells.

BGC823 cells with Hotair overexpression. Meanwhile, GPC5 levels were reduced when down-regulated Hotair expression in GC cells using siRNA. Collectively, these results suggest Hotair directly bind to miR-217 and inhibit its expression, which subsequently increased the expression of GPC-5, a target of miR-217 in GC. These results pinpoint a role of Hotair as a miRNA decoy for miR-217 in GC.

3.7. MiR-217 mimic reversed Hotair functions in GC cells

To further investigated whether Hotair exhibits oncogenic function through regulating miR-217 expression in GC, rescue experiments were conducted. Co-transfected SGC7901 and BGC823 cells with pcDNA3.1-Hotair, and miR-217 mimic or control vector, then examined the change on GC cells proliferative and metastatic capacity. As shown in Fig. 6A and B, overexpression Hotair effectively enhanced the proliferative and metastatic abilities of SGC7901 and BGC823 cells. Moreover, up-regulation the expression of miR-217 alone significantly inhibited the proliferative and metastatic ability of SGC7901 and BGC823 cells, as evidenced by the decrease of cell viability, colonies, migratory and invasive cells number, E-cadherin level and increase N-cadherin, Vimentin, and β-catenin levels. Nevertheless, the influence of pcDNA3.1-Hotair on cell proliferation, apoptosis, migration, invasion, and EMT marker genes expression was effectively rescued by miR-217 mimic in GC cells (Fig. 6A–G). Collectively, abovementioned findings indicated that Hotair controls GC growth and metastasis through acting as a ceRNA to sponge miRNA-217 and directly increased the expression level of GCP5, which subsequently promotes GC progression and metastasis.

4. Discussion

Growing evidence indicated that various differentially expressed lncRNAs play oncogenic or tumor suppressor roles in carcinogenesis [27,28]. For instance, lncRNA UFC1 was a negative prognostic factor in GC and has an oncogenic role through sponging miR-498 and down-regulating Lin28b expression [29]. Xu et al. reported that lncRNA FOXD2-AS1 reinforces tumorigenesis through epigenetically silencing EphB3 mediated EZH2 and LSD1, and serves as a potential biomarker for in GC [30]. Accordingly, in several type cancers, lncRNA Hotair has been found aberrantly high expression and known as a potent oncogene [31]. Ding et al. identified that Estrogen receptor β has oncogenic role in renal cell carcinoma through increasing Hotair level, which subsequently antagonizing several microRNAs, including miR-138, miR-200c, miR-204, or miR-217 to impact various oncogenes, including ADAM9, CCND2, EZH2, VEGFA, VIM, ZEB1, and ZEB2, to promote RCC proliferation and invasion [32]. In addition, aberrant HOTAIR expression Hotair is frequently observed in multiple solid and hematologic malignancies, including prostate cancer [33], lung cancer [34],

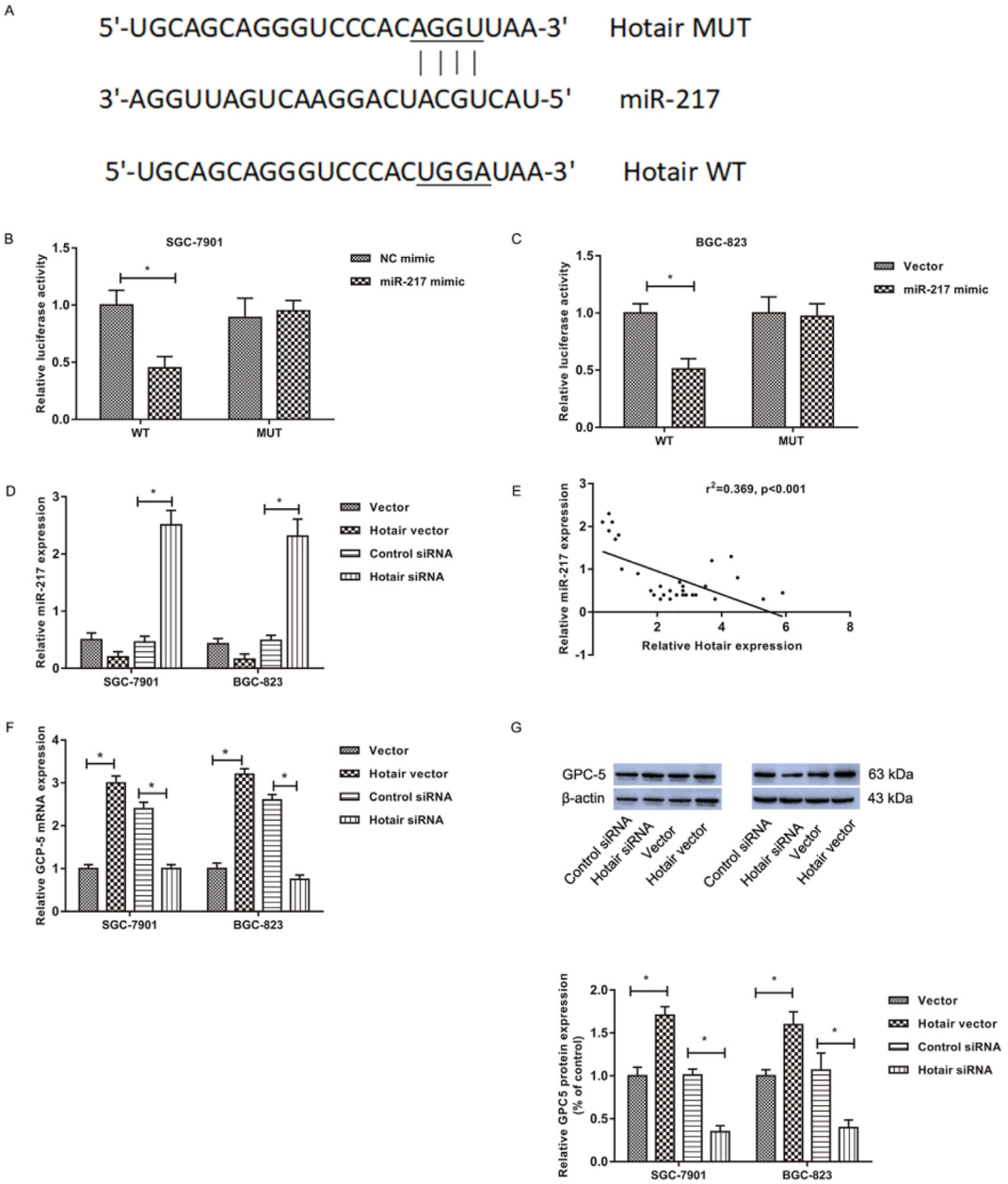

Fig. 5. Hotair facilitates tumor growth and metastasis by sponging miR-217 in GC cells. A. Predicted binding sequence of miR-217 in Hotail. B. After co-transfection GSC7901 and BGC-823 cells with miR-217 mimic and luciferase reporters containing Hotair-Wt or Hotair-Mut transcript, luciferase assay analysis 3'UTR reporter activity. C. qRT-PCR analysis determine the level of miR-217 in cells transfected with control vector, Hotair vector, control siRNA or Hotair siRNA. D. The correlation between Hotair expression and miR-214 level was estimated by using Spearman's rank correlation analysis. E. Following GC cells transfected with empty vector, Hotair vector, siRNA control, Hotair siRNA, the mRNA and protein levels of GPC-5 mRNA were examined by qRT-PCR and western blotting.

hepatocellular carcinoma [35], acute myeloid leukemia (AML) and chronic lymphocytic leukemia [36]. In the present study, we observed that Hotair level was notably up-regulated in GC tissues and GC cell lines, and Hotair level was tightly correlated with the aggressiveness, prognosis, and survival rate of GC patients. In addition, *In vitro* functional experiments demonstrated the oncogenic role of Hotair in promoting cellular proliferation, inhibiting apoptosis, and enhancing metastasis in GC. Knockdown Hotair significantly suppressed cell migration and invasion ability through suppression EMT process. These findings were consistent with previous reports showing that Hotair promotes tumourigenesis in hepatocellular carcinoma [37] and colorectal cancer [38]. Moreover, *in vivo* experiment, ectopic Hotair could remarkably promote GC cell growth, and distant metastasis to liver and lung organs. Thus, we proposed that Hotair might be a potential biomarkers and therapeutic targets in GC.

Recently, a large body of evidence demonstrated that numerous lnRNAs exert their function in tumorgenesis and progression by sponging miRNAs and then subsequently to impact the expression of various oncogenes or tumor suppressor. For instance, lncRNA-GAS5 enhanced the sensitivity of breast cancer cells to tamoxifen through sponging miR-222 and repressing its level, which then reversed the suppression on PTEN [39]. Shan et al. also reported that lncRNA SNHG7 promotes colorectal cells growth and metastasis through sponging miR-216b, and then increased the expression of oncogene, GALNT1 [40]. Intriguingly, Hotair has been found to modulate carcinogenesis and progression through directly targeting miRNAs. Ling et al. revealed that Hotair interacted with EZH2 to inhibit miR-193a level which mediated by the high methylation of H3K27 at miR-193a

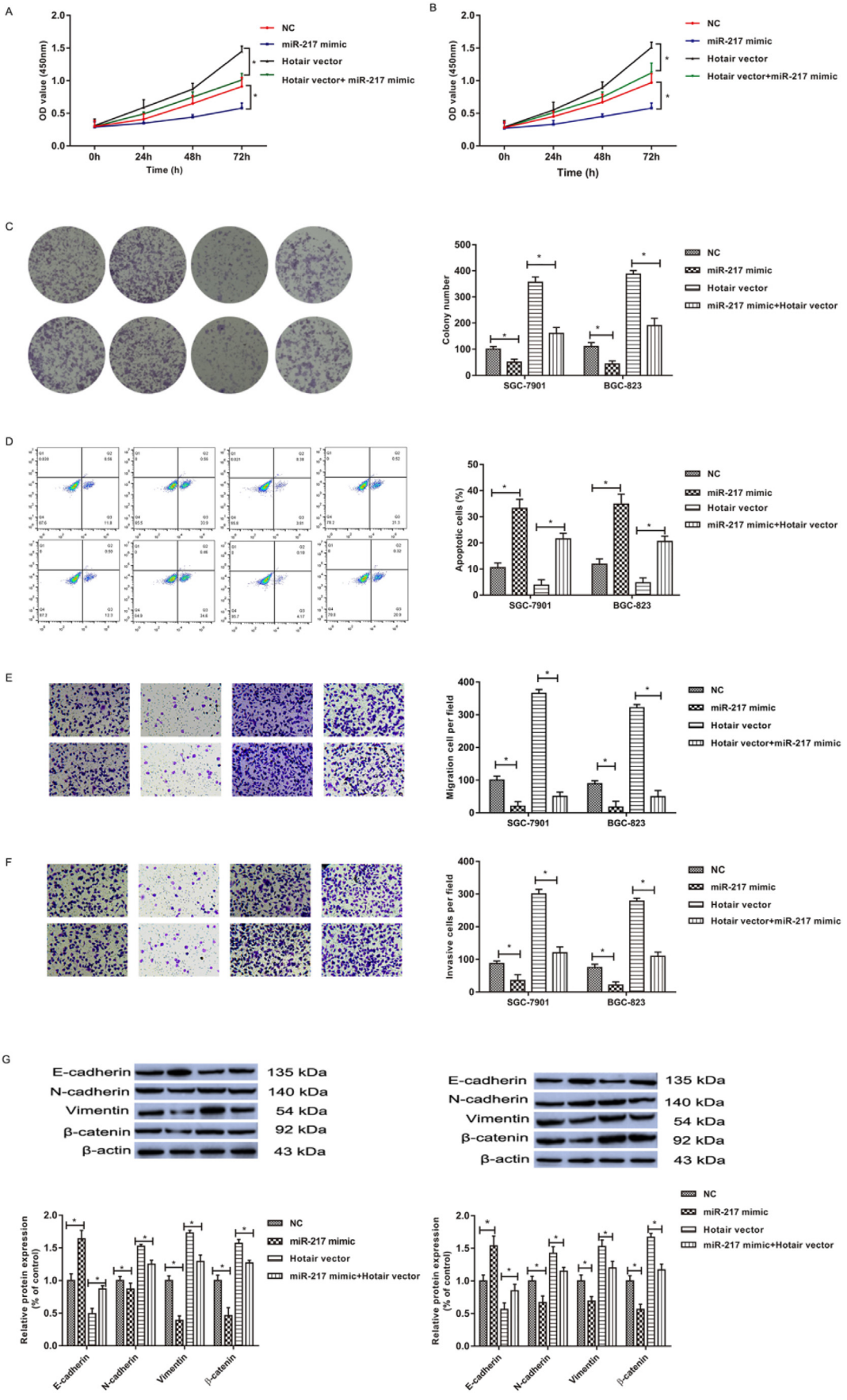

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Fig. 6. MiR-217 mimic abrogated the effect of Hotair overexpression on GC cell proliferation, apoptosis, migration, invasion, and EMT. A. The overexpression of miR-217 was verified by qRT-PCR. B. After, cell growth and proliferation were measured by CCK-8 assay and colony formation assay. C. Flow cytometry analysis cell apoptotic percentage. D. Western blotting analysis the expression levels of cleavd casapse-3, E-cadherin, N-cadherin, vimentin, β-catenin. E.F. After transfection GSC7901 and BGC-823 cells with miR-217 mimic and Hotair vector, cell migration and invasion were determined by using transwell assays. G. Immunofluorescence staining of E-cadherin and N-cadherin expression in GSC7901 and BGC-823, after co-transfection cells with miR-217 mimic and Hotair vector.

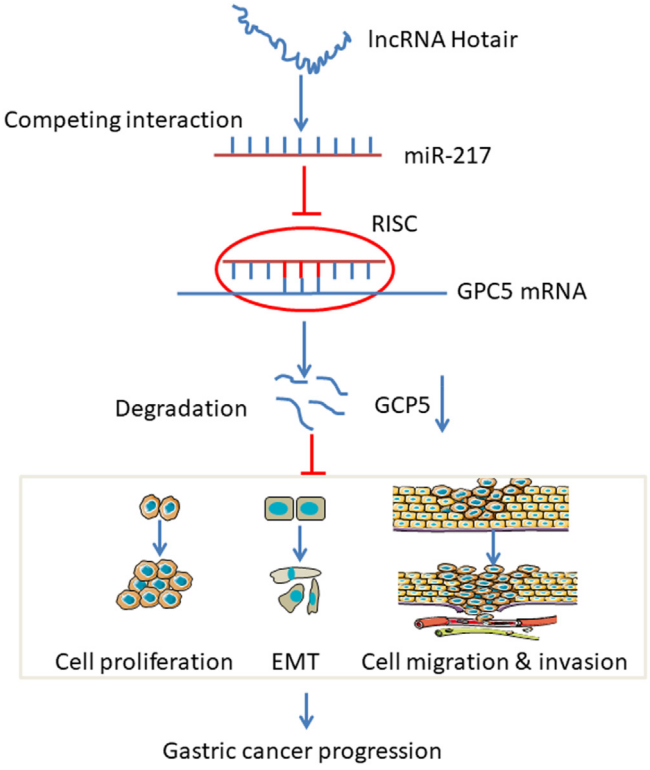

Fig. 7. Schematic diagram for the underlying mechanism of Hotair-mediated promoting Gastric cancer cell growth and metastasis.

promoter in prostate cancer. In this study, we found Hotair could sponge miR-217 and repress its level in GC. There was a negative correlation between the expression of Hotair and miR-217 level, and miR-217 mimic could effectively abrogate the promoting effect of Hotair on GC cells growth and metastasis. Moreover, our previous study has demonstrated miR-217 was down-regulated in GC tissues and cell lines, and ectopic expression miR-217 could effectively suppress cell growth and metastasis by down-regulation GPC5 level [22]. Consistent with previous findings, our results found overexpression Hotair increased GCP5 level, meanwhile, down-regulation Hotair level also cause a decrease in GCP5. Moreover, a recent study by Ding et al. found that Estrogen receptor β promotes renal cell carcinoma progression *via* regulating LncRNA HOTAIR-miR-138/200c/204/217 associated ceRNA network [32]. A large body evidence also confirmed that Hotair exhibits oncogenic role through serving as competing endogenous RNAs and inhibiting targeted miRNAs levels [15,32,38].

5. Conclusions

Collectively, our results suggest Hotair was remarkably up-regulated in GC tissues and promoted cell proliferation, migration, and invasion, EMT and promoted cell evading apoptosis through sponging miR-217 in GC cells. Combined with previous study, these results demonstrate that Hotair/miR-217 level may be a prognostic indicator and Hotair/miR-217/GCP5 axis may be a potential therapeutic target in gastric cancer patients (Fig. 7). This work, as well as related findings from other groups, provides a solid pathophysiological foundation for potential involvement of Hotair/miR-217 in GC.

Acknowledgements

This study was supported by Health Science and Technology Talent Training of Kunming (grant number: 2015-SW (reserve)-01), Medical reserve personnel training program of Yunnan Provincial Health and Family Planning Commission (grant number: H-2017022), The Applied Basic Research Foundation of YunNan Province (grant number: 2017FE468 (-096)), Science Research Foundation of Yunnan Education Bureau (grant number: 2016ZDX069).

Conflict of interest statement

The authors declare that they have no conflict of interest.

Ethical approval

All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

References

[1] J. Ferlay, I. Soerjomataram, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. Parkin, D. Forman, F. Bray, Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012, Int. J. Cancer 136 (5) (2015) E359–E386.
[2] W. Chen, R. Zheng, P.D. Baade, S. Zhang, H. Zeng, F. Bray, A. Jemal, X.Q. Yu, J. He, Cancer statistics in China, 2015, CA Cancer J. Clin. 66 (2) (2016) 115–132.
[3] P. Karimi, F. Islami, S. Anandasabapathy, N.D. Freedman, F. Kamangar, Gastric cancer: descriptive epidemiology, risk factors, screening, and prevention, Cancer Epidemiol. Biomark. Prev. 23 (5) (2014) 700–713.
[4] L. Shen, Y. Shan, H. Hu, T. Price, B. Sirohi, K. Yeh, Y. Yang, T. Sano, H. Yang, X. Zhang, et al., Management of gastric cancer in Asia: resource-stratified guidelines, Lancet Oncol. 14 (12) (2013) e535–e547.
[5] A. Thiel, A. Ristimaki, Gastric cancer: basic aspects, Helicobacter 17 Suppl 1 (2012) 26–29.
[6] A. Fatica, I. Bozzoni, Long non-coding RNAs: new players in cell differentiation and development, Nat. Rev. Genet. 15 (1) (2014) 7–21.
[7] M. Huarte, The emerging role of lncRNAs in cancer, Nat. Med. 21 (11) (2015) 1253–1261.
[8] X. Song, Y. Sun, A. Garen, Roles of PSF protein and VL30 RNA in reversible gene regulation, Proc. Natl. Acad. Sci. U. S. A. 102 (34) (2005) 12189–12193.
[9] J. Lee, H. Kim, J.E. Lee, S.J. Shin, S. Oh, S. Kwon, H. Kim, Y.Y. Choi, M.A. White, S. Paik, et al., Selective cytotoxicity of the NAMPT inhibitor FK866 toward gastric cancer cells with markers of the epithelial-mesenchymal transition, due to loss of NAPRT, Gastroenterology 155 (3) (2018) 799–814.
[10] Y. Yin, A.M. Grabowska, P.A. Clarke, E. Whelband, K. Robinson, R.H. Argent, A. Tobias, R. Kumari, J.C. Atherton, S.A. Watson, Helicobacter pylori potentiates epithelial:mesenchymal transition in gastric cancer: links to soluble HB-EGF, gastrin and matrix metalloproteinase-7, Gut 59 (8) (2010) 1037–1045.
[11] Q. Wu, S. Xiang, J. Ma, P. Hui, T. Wang, W. Meng, M. Shi, Y. Wang, Long non-coding RNA CASC15 regulates gastric cancer cell proliferation, migration and epithelial mesenchymal transition by targeting CDKN1A and ZEB1, Mol. Oncol. 12 (6) (2018) 799–813.
[12] K. Yan, J. Tian, W. Shi, H. Xia, Y. Zhu, LncRNA SNHG6 is associated with poor prognosis of gastric cancer and promotes cell proliferation and EMT through epigenetically silencing p27 and sponging miR-101-3p, Cell. Physiol. Biochem. 42 (3) (2017) 999–1012.
[13] R.A. Gupta, N. Shah, K.C. Wang, J. Kim, H.M. Horlings, D.J. Wong, M.C. Tsai, T. Hung, P. Argani, J.L. Rinn, et al., Long non-coding RNA HOTAIR reprograms chromatin state to promote cancer metastasis, Nature 464 (7291) (2010) 1071–1076.
[14] T. Yang, X. He, A. Chen, K. Tan, X. Du, LncRNA HOTAIR contributes to the malignancy of hepatocellular carcinoma by enhancing epithelial-mesenchymal transition via sponging miR-23b-3p from ZEB1, Gene 670 (2018) 114–122.
[15] P. Dasgupta, P. Kulkarni, S. Majid, V. Shahryari, Y. Hashimoto, N.S. Bhat, M. Shiina, G. Deng, S. Saini, Z.L. Tabatabai, et al., MicroRNA-203 inhibits long noncoding RNA HOTAIR and regulates tumorigenesis through epithelial-to-mesenchymal transition pathway in renal cell carcinoma, Mol. Cancer Ther. 17 (5) (2018) 1061–1069.
[16] J.T. Li, L.F. Wang, Y.L. Zhao, T. Yang, W. Li, J. Zhao, F. Yu, L. Wang, Y.L. Meng, N.N. Liu, et al., Nuclear factor of activated T cells 5 maintained by Hotair suppression of miR-568 upregulates S100 calcium binding protein A4 to promote breast cancer metastasis, Breast Cancer Res. 16 (5) (2014) 454.

[17] C. Da, L. Wu, Y. Liu, R. Wang, R. Li, Effects of irradiation on radioresistance, HOTAIR and epithelial-mesenchymal transition/cancer stem cell marker expression in esophageal squamous cell carcinoma, Oncol. Lett. 13 (4) (2017) 2751–2757.
[18] L. Salmena, L. Poliseno, Y. Tay, L. Kats, P.P. Pandolfi, A ceRNA hypothesis: the Rosetta Stone of a hidden RNA language? Cell 146 (3) (2011) 353–358.
[19] W.C. Liang, W.M. Fu, C.W. Wong, Y. Wang, W.M. Wang, G.X. Hu, L. Zhang, L.J. Xiao, D.C. Wan, J.F. Zhang, et al., The lncRNA H19 promotes epithelial to mesenchymal transition by functioning as miRNA sponges in colorectal cancer, Oncotarget 6 (26) (2015) 22513–22525.
[20] W. Lu, H. Zhang, Y. Niu, Y. Wu, W. Sun, H. Li, J. Kong, K. Ding, H.M. Shen, H. Wu, et al., Long non-coding RNA linc00673 regulated non-small cell lung cancer proliferation, migration, invasion and epithelial mesenchymal transition by sponging miR-150-5p, Mol. Cancer 16 (1) (2017) 118.
[21] S.P. Li, H.X. Xu, Y. Yu, J.D. He, Z. Wang, Y.J. Xu, C.Y. Wang, H.M. Zhang, R.X. Zhang, J.J. Zhang, et al., LncRNA HULC enhances epithelial-mesenchymal transition to promote tumorigenesis and metastasis of hepatocellular carcinoma via the miR-200a-3p/ZEB1 signaling pathway, Oncotarget 7 (27) (2016) 42431–42446.
[22] H. Wang, X. Dong, X. Gu, R. Qin, H. Jia, J. Gao, The MicroRNA-217 functions as a potential tumor suppressor in gastric cancer by targeting GPC5, PLoS One 10 (6) (2015) e0125474.
[23] K. Zhou, C. Zhang, H. Yao, X. Zhang, Y. Zhou, Y. Che, Y. Huang, Knockdown of long non-coding RNA NEAT1 inhibits glioma cell migration and invasion via modulation of SOX2 targeted by miR-132, Mol. Cancer 17 (1) (2018) 105.
[24] Z. Li, X. Yu, J. Liang, W.K. Wu, J. Yu, J. Shen, Leptin downregulates aggrecan through the p38-ADAMST pathway in human nucleus pulposus cells, PLoS One 9 (10) (2014) e109595.
[25] Q. Hong, O. Li, W. Zheng, W.Z. Xiao, L. Zhang, D. Wu, G.Y. Cai, J.C. He, X.M. Chen, LncRNA HOTAIR regulates HIF-1alpha/AXL signaling through inhibition of miR-217 in renal cell carcinoma, Cell Death Dis. 8 (5) (2017) e2772.
[26] L. Wang, F. Yang, L.T. Jia, A.G. Yang, Missing links in epithelial-mesenchymal transition: long non-coding RNAs enter the arena, Cell. Physiol. Biochem. 44 (4) (2017) 1665–1680.
[27] P.F. Li, S.C. Chen, T. Xia, X.M. Jiang, Y.F. Shao, B.X. Xiao, J.M. Guo, Non-coding RNAs and gastric cancer, World J. Gastroenterol. 20 (18) (2014) 5411–5419.
[28] S. Chandra Gupta, Y. Nandan Tripathi, Potential of long non-coding RNAs in cancer patients: from biomarkers to therapeutic targets, Int. J. Cancer 140 (9) (2017) 1955–1967.
[29] X. Zhang, W. Liang, J. Liu, X. Zang, J. Gu, L. Pan, H. Shi, M. Fu, Z. Huang, Y. Zhang, et al., Long non-coding RNA UFC1 promotes gastric cancer progression by regulating miR-498/Lin28b, J. Exp. Clin. Cancer Res. 37 (1) (2018) 134.
[30] T.P. Xu, W.Y. Wang, P. Ma, Y. Shuai, K. Zhao, Y.F. Wang, W. Li, R. Xia, W.M. Chen, E.B. Zhang, et al., Upregulation of the long noncoding RNA FOXD2-AS1 promotes carcinogenesis by epigenetically silencing EphB3 through EZH2 and LSD1, and predicts poor prognosis in gastric cancer, Oncogene 37 (36) (2018) 5020–5036.
[31] Q. Tang, S. Hann, HOTAIR: an oncogenic long non-coding RNA in human Cancer, Cell. Physiol. Biochem. 47 (3) (2018) 893–913.
[32] J. Ding, C.R. Yeh, Y. Sun, C. Lin, J. Chou, Z. Ou, C. Chang, J. Qi, S. Yeh, Estrogen receptor beta promotes renal cell carcinoma progression via regulating LncRNA HOTAIR-miR-138/200c/204/217 associated CeRNA network, Oncogene 37 (37) (2018) 5037–5053.
[33] Y.T. Chang, T.P. Lin, J.T. Tang, M. Campbell, Y.L. Luo, S.Y. Lu, C.P. Yang, T.Y. Cheng, C.H. Chang, T.T. Liu, et al., HOTAIR is a REST-regulated lncRNA that promotes neuroendocrine differentiation in castration resistant prostate cancer, Cancer Lett. 433 (2018) 43–52.
[34] X.H. Liu, Z.L. Liu, M. Sun, J. Liu, Z.X. Wang, W. De, The long non-coding RNA HOTAIR indicates a poor prognosis and promotes metastasis in non-small cell lung cancer, BMC Cancer 13 (2013) 464.
[35] Z. Yang, L. Zhou, L.M. Wu, M.C. Lai, H.Y. Xie, F. Zhang, S.S. Zheng, Overexpression of long non-coding RNA HOTAIR predicts tumor recurrence in hepatocellular carcinoma patients following liver transplantation, Ann. Surg. Oncol. 18 (5) (2011) 1243–1250.
[36] M. Isin, E. Ozgur, G. Cetin, N. Erten, M. Aktan, U. Gezer, N. Dalay, Investigation of circulating lncRNAs in B-cell neoplasms, Clin. Chim. Acta 431 (2014) 255–259.
[37] W.M. Fu, X. Zhu, W.M. Wang, Y.F. Lu, B.G. Hu, H. Wang, W.C. Liang, S.S. Wang, C.H. Ko, M.M. Waye, et al., Hotair mediates hepatocarcinogenesis through suppressing miRNA-218 expression and activating P14 and P16 signaling, J. Hepatol. 63 (4) (2015) 886–895.
[38] Z. Xiao, Z. Qu, Z. Chen, Z. Fang, K. Zhou, Z. Huang, X. Guo, Y. Zhang, LncRNA HOTAIR is a prognostic biomarker for the proliferation and chemoresistance of colorectal cancer via MiR-203a-3p-mediated Wnt/ss-catenin signaling pathway, Cell. Physiol. Biochem. 46 (3) (2018) 1275–1285.
[39] J. Gu, Y. Wang, X. Wang, D. Zhou, C. Shao, M. Zhou, Z. He, Downregulation of lncRNA GAS5 confers tamoxifen resistance by activating miR-222 in breast cancer, Cancer Lett. 10 (434) (2018) 1–10.
[40] Y. Shan, J. Ma, Y. Pan, J. Hu, B. Liu, L. Jia, LncRNA SNHG7 Sponges miR-216b to Promote Proliferation and Liver Metastasis of Colorectal Cancer Through Upregulating GALNT1, 9(7) (2018), p. 722.