Research Paper

HOXC8 mediates osteopontin expression in gastric cancer cells

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Abstract

Genes of the homeobox (HOX) family encode transcription factors, which play a role in cancer progression. However, their role in gastric cancer has not been adequately evaluated. Herein, we evaluated the genetic changes and mRNA of target genes of the HOX family in gastric cancer patients using publicly available online datasets. We found that *HOXC8* was amplified in gastric cancer tissues, and mRNA expression levels were significantly associated with tumor status ($P=0.044$) and poor overall survival ($P<0.01$). *HOXC8* knockdown significantly reduced the viability of gastric cancer cell lines. *HOXC8* modulated the expression of secreted phosphoprotein 1 (SPP1, osteopontin) and phosphorylation of AKT/ERK in gastric cancer cells. Survival analysis demonstrated a decrease in overall survival rates among the high *HOXC8*/high *SPP1* expression group compared with the low *HOXC8*/low *SPP1* expression group. In conclusion, *HOXC8* may be an independent prognostic factor and serve as a useful predictive biomarker for gastric cancer.

Keywords: *HOXC8*, osteopontin, *SPP1*, and gastric cancer

Introduction

The survival and prognosis of cancer patients are associated with stage status development. Gastric cancer is the sixth most common malignant cancer (1.09 million cases in 2020, World Health Organization) and the leading cause of cancer-related death in the world [1-4]. Among the deaths in Taiwan in 2020, 50,161 were due to cancers, 2,339 (5.2%) of which occurred from gastric cancer (from the Ministry of Health and Welfare of Taiwan). Unfortunately, the lack of highly sensitive and specific biomarkers to diagnose gastric cancer leads to most patients being in the advanced stage before diagnosis [5-7]. Therefore, investigating and improving the diagnostic sensitivity of cancer biomarkers for early-stage tumors is beneficial for improving the survival rate of gastric patients.

The human homeobox (HOX) genes have four chromosomal clusters and 39 HOX genes: *HOXA* (chromosome 7), *HOXB* (chromosome 17), *HOXC* (chromosome 12), and *HOXD* (chromosome 2) [8]. The HOX genes encode transcription factors that are involved in a number of biological processes, including embryonic development, cell proliferation, and differentiation, among others [9, 10]. There has been substantial evidence of aberrant *HOX* gene expression in multiple cancers such as prostate, pancreatic, lung, and breast cancer [8, 11-13]. Numerous studies have shown that diverse HOX genes can either enhance or inhibit the cancer progression process on their abnormal expression in different organs. For example, the HOXB gene is a prognostic factor in breast cancer [14], HOXA13 expression impaired the chemotherapy in gastric cancer cells [15], HOXB5 promotes metastasis in

hepatocellular carcinoma [16] and HOXA13, HOXD13, and HOXC6 were promoted colorectal cancer [17-19]. Furthermore, lymphoblastic leukemia with mixed-lineage leukemia translocations shows increased expression of *HOXA4*, *HOXA5*, *HOXA7*, *HOXA9,* and *HOXB5*, which is related to poor prognosis [20-24]. *HOXC4-6* and *HOXC8* are upregulated in primary tumor and cancer cell lines and are not normally expressed in normal prostate tissues or cell lines [25, 26]. HOX protein can modulate other proteins to enhance or repress gene expression [27] and it also can be expressed in cancer stem cells [28]. In gastric cancer and HOXC6 promotes invasion ability [29]. However, downstream targets of HOX genes have still not been fully identified.

Secreted phosphoprotein 1 (SPP1), also known as osteopontin (OPN) protein was upregulated in primary and metastatic lesions of gastric cancer, which indicates that OPN may play a role in gastric cancer [30, 31]. OPN was first described as a glyco-phosphoprotein that is secreted from malignant epithelial cells [32]. The functions of OPN in cancer progression, include cell adhesion, chemotaxis, invasion, migration, and the anchorage-independent growth of tumor cells [33]. A previous study showed that OPN was downregulated in *HOXC8*-silenced cells [11]. In our study, the highest expression of *HOXC8* was observed in gastric cancer tissues. However, the mechanism by which *HOXC8* modulates OPN expression and regulates the malignant phenotype of gastric cancer cells has not been clearly elucidated.

Materials and Methods

In silico mRNA profiles and Kaplan–Meier analysis of *HOXC8*

HOXC8 mRNA expression in stomach adeno-carcinoma tissues was determined by using The Cancer Genome Atlas (TCGA) dataset and TNMplot (https://tnmplot.com/analysis/). Kaplan–Meier analysis (overall survival) was performed using a publicly available gastric cancer microarray dataset (http://kmplot.com/analysis/).

Cell lines and culture conditions

TSGH, AZ521, and HR cell lines (human gastric carcinoma) from Dr. Kuo-Wang Tsai at Taipei Tzu Chi Hospital in Taiwan. Cells were cultured in Dulbecco's Modified Eagle Medium with 10% fetal bovine serum and 1% penicillin-streptomycin-glutamine.

Lentivirus infection

Lentivirus vector control (pLKO-1-shLuc967) and shHOXC8 shRNA viral supernatant (TRCN00 00000019564, TRCN0000019565) were purchased from the National RNAi Core Facility (Taipei, Taiwan). The viral supernatants were used to infect AZ521 or HR cell lines with 8 μg/mL of polybrene at 72 h. After infection, cells were selected by using 2 μg/mL of puromycin.

Real-time-polymerase chain reaction (PCR)

Total RNA was extracted using TRI Reagent (Sigma-Aldrich, #T9424), cDNA was synthesized by TaKaRa PrimeScript™ RT reagent Kit (Cat. #RR037A), and PCR reactions were conducted using SYBR system (PCR Biosystems qPCRBIO SyGreen Mix Lo-ROX). The following primer sequences were used: *HOXC8*, forward: 5'TCAAAACTCGTCTCC CAGCC-3'; *HOXC8*, reverse: 5' TTCCAAGGT CTGATACCGGC-3'; *SPP1*, forward: 5'ATGATG GCCGAGGTGATAGTG; *SPP1*, reverse: GAGGTG ATGTCCTCGTCTGTAGC; 5'*ACTB*, forward: 5' AGAAAATCTGGCACCACACC-3' and *ACTB*, reverse: 5' AGAGGCGTACAGGGATAGCA-3'.

Growth curve assay

Cells (2,000 cells/well for AZ-521/shluc, AZ-521/shHOXC8-1, AZ-521/shHOXC8-2, HR/shluc, HR/shHOXC8-1, and HR/shHOXC8-2) were seeded in a 96-well plate for 24–72 h (incubated at 37°C with 5% CO_2). The cell growth curve was determined using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Colony formation assay

The stable lines (1,000 cells/well) were plated in a 6-well plate. After a 7-day incubation at 37°C with 5% CO_2, the cells were fixed and stained using crystal violet. The number of colonies was counted using the NIH Image J software.

Western blot analysis

Western blot analysis was performed using the following primary antibodies: anti-OPN (IBL, #18625,1:1000), anti-pAKT (Cell Signaling, #4060, 1:1000), anti-AKT (Cell Signaling, #4691,1:1000), anti-pERK (Cell Signaling, #9101,1:1000), anti-ERK (Cell Signaling, #9102,1:1000), and β-actin (Sigma-Aldrich, #A5441, 1:5000).

Results

In silico genetic alterations of *HOXC* family members in stomach adenocarcinoma

cBioPortal was used to examine the genetic alterations of the *HOXC* family in stomach adenocarcinoma (STAD) patients. *HOXC4~HOXC13* had a 1.5-2.3% amplification, and *HOXC9* had a 4% mutation ratio in STAD patients (Figure 1A-B). Further analyzing the frequency of co-occurrence of

the *HOXC* family genetically altered in the same tumor tissues. Co-occurrence analysis of the STAD sample identified the *HOXC* family genetically altered significant co-occurrence in the same tumor sample (Figure 1C and Table 1). Previous studies showed several members of the *HOXC* family have been well-investigated for their role in gastric cancer [34, 35]. Moreover, *HOXC8* knockdown reduced cell proliferation in gastric cancer [36]. However, the molecular mechanism of its action remains unclear. Therefore, in this study, we focused on *HOXC8* to further investigate gastric cancer.

The HOXC8 expression level was significantly increased and correlates with poor survival rates in STAD patients

We next evaluated the mRNA levels of *HOXC8* in multiple cancer types using TNMplot, which showed that *HOXC8* was upregulated in many cancer types, including STAD (Figure 2A). We further analyzed the TCGA dataset to understand the clinical prognostic value of *HOXC8* in STAD patients. Expression profiles were downloaded from TCGA, including 408 STAD tissues and 36 adjacent normal tissues. As shown in Figure 2B, the expression level of *HOXC8* was significantly increased in STAD patients compared to adjacent normal tissues. The expression levels of *HOXC8* in human STAD tissues were further evaluated in 95 patients with stage I-II STAD and 272 with stage III-IV STAD. *HOXC8* expression analysis was based on the expression of *HOXC8* mRNA. Using the clarified expression criteria, we classified patients into the *HOXC8*-low and *HOXC8*-high groups. The data showed that *HOXC8* expression was significantly associated with tumor stage (P=0.044) (Table 2). Next, we detected the overall survival rate of *HOXC8* in a clinical cohort using Kaplan–Meier analysis. A high level of *HOXC8* expression correlated with the overall survival rate in patients with gastric cancer (Figure 2C). However, the detailed role of *HOXC8* in STAD progression remains unclear.

Table 1. Co-occurring of HOXC family genetic alterations in stomach adenocarcinoma.

| A	B	Neither	A Not B	B Not A	Both	Log2 Odds Ratio	p-Value	q-Value	Tendency
HOXC5	HOXC10	1385	9	10	24	>3	<0.001	<0.001	Co-occurrence
HOXC4	HOXC8	1404	4	3	17	>3	<0.001	<0.001	Co-occurrence
HOXC4	HOXC6	1399	3	8	18	>3	<0.001	<0.001	Co-occurrence
HOXC5	HOXC9	1388	13	7	20	>3	<0.001	<0.001	Co-occurrence
HOXC6	HOXC8	1399	9	3	17	>3	<0.001	<0.001	Co-occurrence
HOXC8	HOXC9	1398	3	10	17	>3	<0.001	<0.001	Co-occurrence
HOXC4	HOXC9	1397	4	10	17	>3	<0.001	<0.001	Co-occurrence
HOXC9	HOXC10	1386	8	15	19	>3	<0.001	<0.001	Co-occurrence
HOXC5	HOXC8	1392	16	3	17	>3	<0.001	<0.001	Co-occurrence
HOXC4	HOXC11	1402	6	5	15	>3	<0.001	<0.001	Co-occurrence
HOXC4	HOXC5	1391	4	16	17	>3	<0.001	<0.001	Co-occurrence
HOXC4	HOXC10	1390	4	17	17	>3	<0.001	<0.001	Co-occurrence
HOXC6	HOXC9	1392	9	10	17	>3	<0.001	<0.001	Co-occurrence
HOXC11	HOXC12	1395	4	13	16	>3	<0.001	<0.001	Co-occurrence
HOXC10	HOXC11	1390	18	4	16	>3	<0.001	<0.001	Co-occurrence
HOXC8	HOXC10	1390	4	18	16	>3	<0.001	<0.001	Co-occurrence
HOXC6	HOXC11	1397	11	5	15	>3	<0.001	<0.001	Co-occurrence
HOXC8	HOXC11	1402	6	6	14	>3	<0.001	<0.001	Co-occurrence
HOXC12	HOXC13	1387	12	12	17	>3	<0.001	<0.001	Co-occurrence
HOXC5	HOXC6	1386	16	9	17	>3	<0.001	<0.001	Co-occurrence
HOXC6	HOXC10	1385	9	17	17	>3	<0.001	<0.001	Co-occurrence
HOXC4	HOXC12	1393	6	14	15	>3	<0.001	<0.001	Co-occurrence
HOXC5	HOXC13	1383	16	12	17	>3	<0.001	<0.001	Co-occurrence
HOXC10	HOXC13	1382	17	12	17	>3	<0.001	<0.001	Co-occurrence
HOXC9	HOXC11	1395	13	6	14	>3	<0.001	<0.001	Co-occurrence
HOXC8	HOXC12	1393	6	15	14	>3	<0.001	<0.001	Co-occurrence
HOXC8	HOXC13	1393	6	15	14	>3	<0.001	<0.001	Co-occurrence
HOXC11	HOXC13	1393	6	15	14	>3	<0.001	<0.001	Co-occurrence
HOXC6	HOXC12	1388	11	14	15	>3	<0.001	<0.001	Co-occurrence
HOXC4	HOXC13	1392	7	15	14	>3	<0.001	<0.001	Co-occurrence
HOXC10	HOXC12	1381	18	13	16	>3	<0.001	<0.001	Co-occurrence
HOXC5	HOXC11	1389	19	6	14	>3	<0.001	<0.001	Co-occurrence
HOXC6	HOXC13	1387	12	15	14	>3	<0.001	<0.001	Co-occurrence
HOXC9	HOXC12	1386	13	15	14	>3	<0.001	<0.001	Co-occurrence
HOXC9	HOXC13	1386	13	15	14	>3	<0.001	<0.001	Co-occurrence
HOXC5	HOXC12	1380	19	15	14	>3	<0.001	<0.001	Co-occurrence
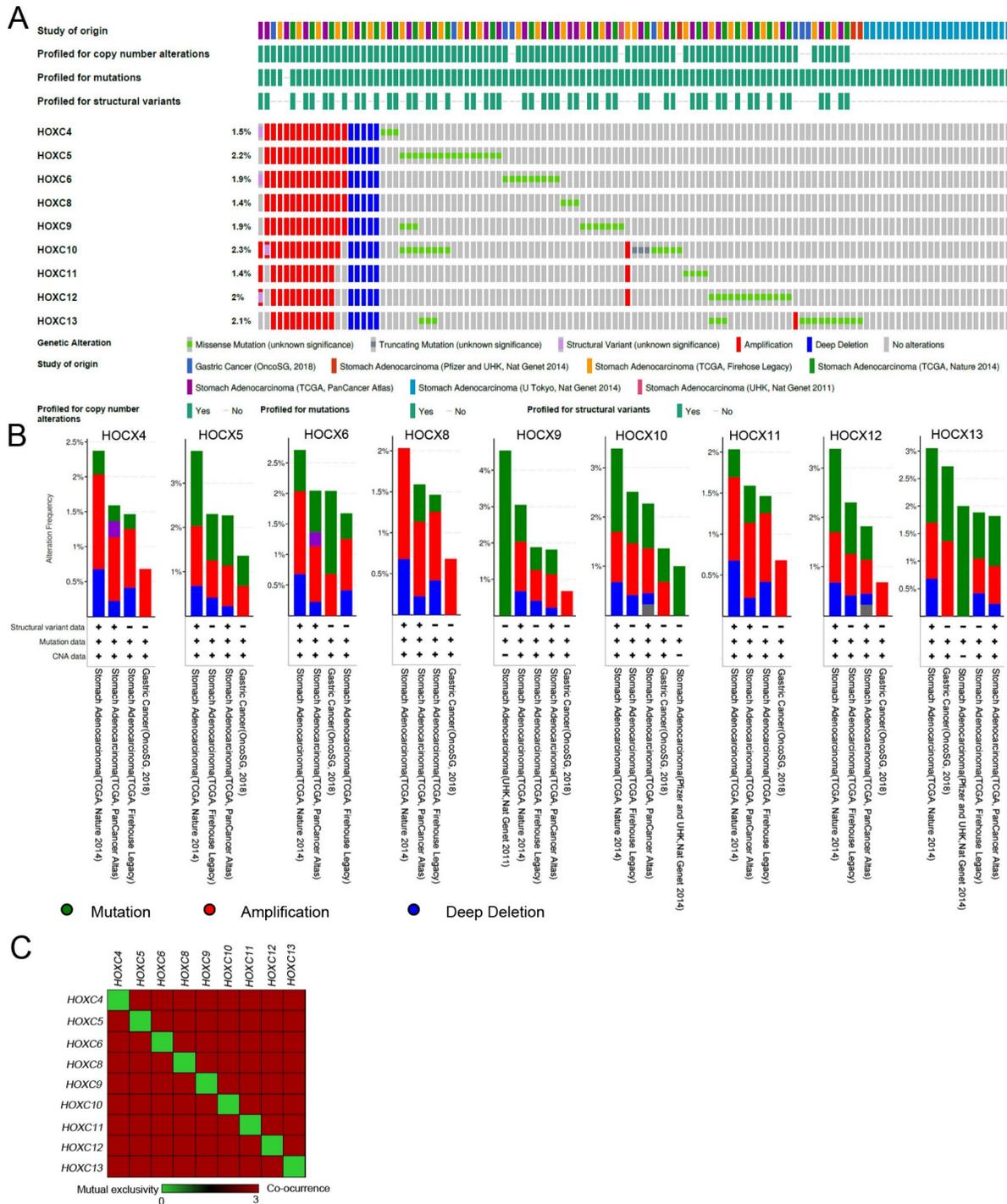
Figure 1. HOXC family amplification in stomach adenocarcinoma (STAD). (A) Oncoprint showing *HOXC4*, *HOXC5*, *HOXC6*, *HOXC8*, *HOXC9*, *HOXC10*, *HOXC11*, *HOXC12*, and *HOXC13* in STAD patients. (B) Cancer type summary of *HOXC4*, *HOXC5*, *HOXC6*, *HOXC8*, *HOXC9*, *HOXC10*, *HOXC11*, *HOXC12*, and *HOXC13* by different STAD cohorts. (C) Co-occurrence analysis of *HOXC* family members in STAD tumors.

HOXC8 knockdown reduced proliferation and colony formation in gastric cancer cells

According to the clinical data, we found that *HOXC8* expression was associated with tumor size (Table 2). Therefore, we evaluated the effect of HOXC8 on proliferation and colony formation in gastric cancer cells. First, we examined the expression

levels of *HOXC8* in gastric cancer cell lines. The results revealed that *HOXC8* was upregulated in AZ521 and HR cancer cell lines compared with that in a TGCH cell line (Figure 3A). Next, we evaluated the effect of *HOXC8* on cell viability and colony formation in gastric cancer cells. *HOXC8* knockdown significantly reduced the cell growth and colony formation in AZ521 and HR cells (Figure 3B-D).

Table 2. Association of *HOXC8* expression with clinicopathological characteristics in 367 gastric cancer patients.

Variables	Item	Patient No.	HOXC8 Low No. (%)	High No. (%)	p value*
			126	241	
Sex	Female		41	148	0.304
	Male		85	93	
Stage	I/II		57	110	1.000
	III/IV		69	131	
T status	T1/T2		41	54	0.044*
	T3/T4		85	187	
M status	Negative		118	223	0.831
	Positive		8	18	
Lymph node status	Negative		36	81	0.347
	Positive		90	160	

*P value < 0.05 was considered statistically significant (chi-square test for categorical variables).
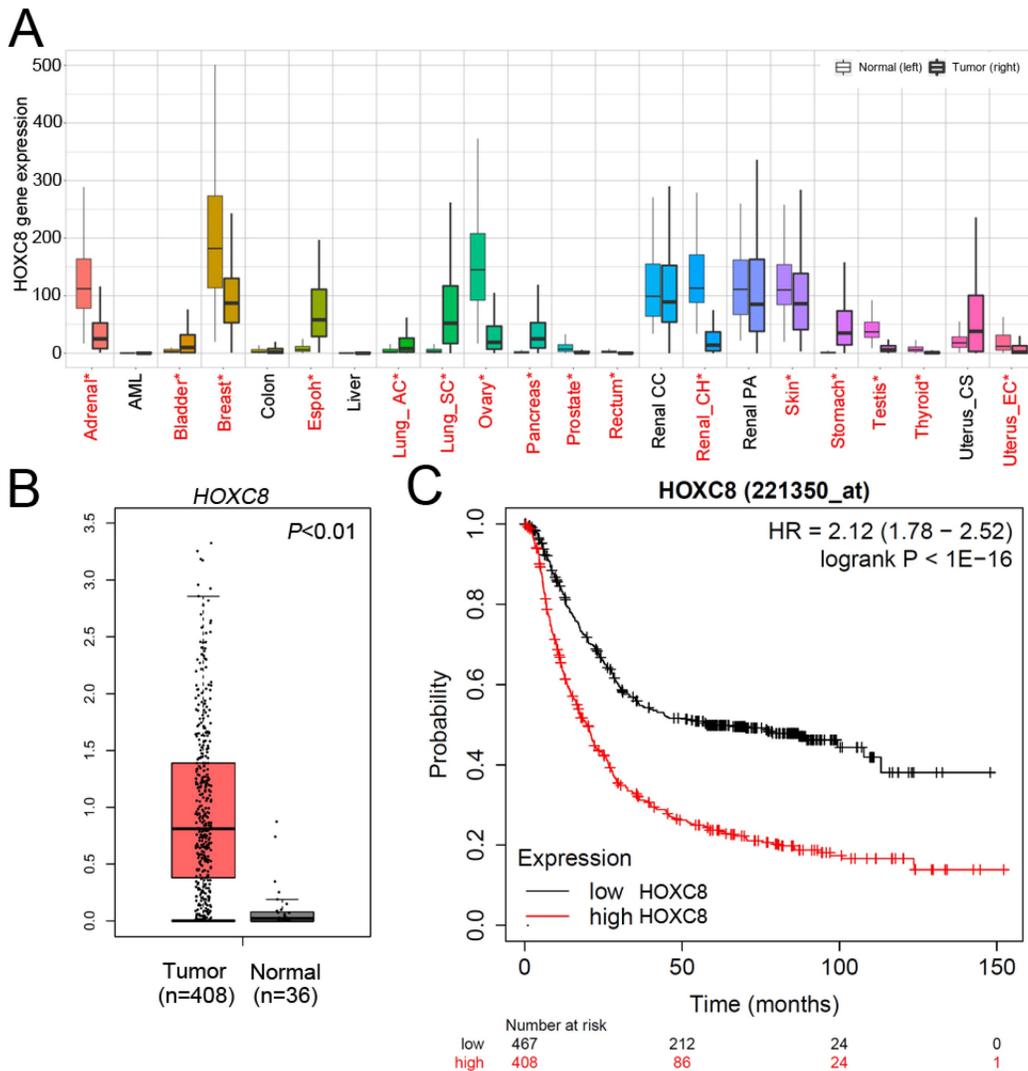
Figure 2. *HOXC8* is overexpressed in gastric cancer and is correlated with poor survival. (A) *HOXC8* mRNA expression in multiple cancer types (TNMplot). (B) The expression of *HOXC8* is significantly upregulated in STAD tumors compared to that in adjacent normal tissue, as determined using TCGA dataset. (C) Kaplan–Meier graph of overall survival in publicly available gastric cancer microarray datasets, stratified according to *HOXC8* expression (Kaplan-Meier Plotter).

HOXC8 downregulated the inhibitory OPN pathway in gastric cancer cells

The *SPP1* gene contains a *HOXC8* responsive element in the promoter region [37]. We also used the pathway commons database (https://www.pathwaycommons.org/) to identify the associated molecular targets of HOXC8. As show in Figure 4A,

HOXC8 interaction with 24 genes, including SMAD4, PRDM4, GMNN, ZFP90, PBX2, RBPMS, PBX1, PDE4DIP, PLA2G10, ABL1, C1orf109, KRTAP12-1, LHX2, TEKT4, LHX3, TRIM42, FNTB, BLZF1, CYSRT1, SMAD1, KPRP, ZRANB1 and FOXO1. In this study, we focus on SMAD4 which has been reported to mediate OPN expression in cancer cells [38]. We examined the correlation between *HOXC8* and *SMAD4* in patients with STAD using the TIMER. We found that *HOXC8* levels was significantly negatively correlated with *SMAD4* levels in STAD patients (Figure 4B). *SMAD4* upregulated level associated prolong overall survival in gastric cancer patients (Figure 4C). We further confirmed whether there was *HOXC8*-mediated *SPP1* expression in gastric cancer cells. The results showed that the expression of *SPP1* was significantly reduced in gastric cancer cells (Figure 4D). We then examined *SPP1* expression in gastric cancer patients using the TCGA database. *SPP1* was upregulated in STAD tissues compared to normal tissues (Figure 4E). Although *SPP1* expression has not significantly associated with overall survival in gastric cancer patients (Figure 4F). The high *HOXC8*/high *SPP1* group was associated with shorter overall survival than the low *HOXC8*/low *SPP1* group was among gastric cancer patients (Figure 4G). Moreover, OPN modulated the phosphorylation of AKT and ERK in lung cancer cells [39]. *HOXC8* knockdown significantly reduced the phosphorylation of AKT and ERK in gastric cancer cells (Figure 4H).
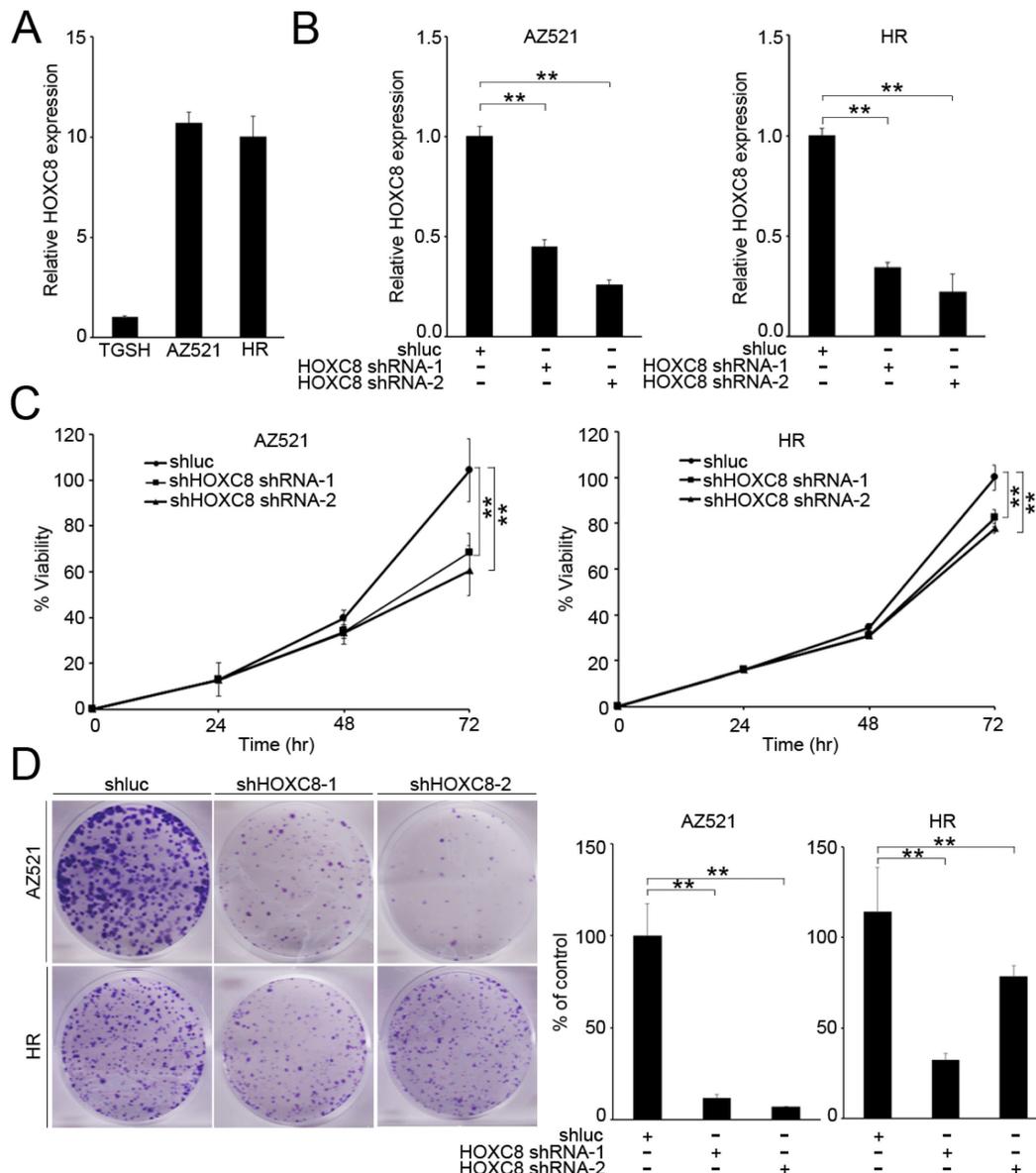

Figure 3. *HOXC8* knockdown inhibits cell proliferation. (A) Real-time polymerase chain reaction (PCR) analysis of *HOXC8* expression in gastric cancer cell lines. (B) Real-time PCR analysis of *HOXC8* expression after infection with shRNA. (C) Effect of *HOXC8* knockdown on proliferation of AZ521 and HR cell lines. (D) Downregulation of *HOXC8* reduced colony formation of gastric cancer cells.
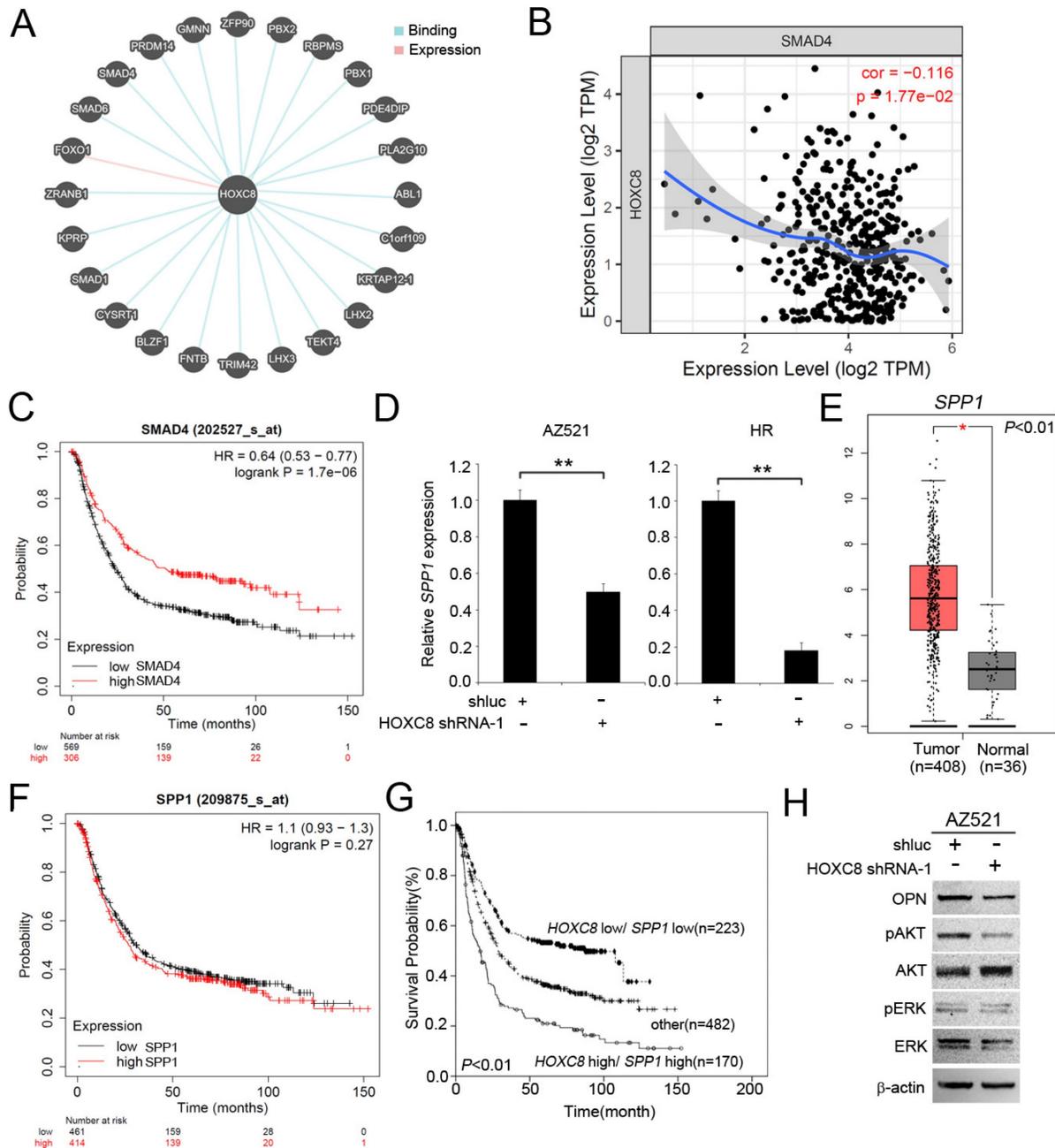
Figure 4. *HOXC8* mediates the OPN pathway in gastric cancer cells. (A) Putative binding interaction targets of HOXC8 were identified from Pathway Commons. (B) Analysis of the correlation between *HOXC8* and *SMAD4* mRNA expression by using TIMER. (C) Kaplan–Meier analysis of overall survival, according to *SMAD4* mRNA expression using publicly available gastric cancer microarray datasets (Kaplan-Meier Plotter). (D) Expression of *SPP1* mRNA in *HOXC8* knockdown cells compared with shluc control. (E) Relative mRNA levels of *SPP1* in stomach adenocarcinoma tissues (The Cancer Genome Atlas dataset). (F) According to *SPP1* mRNA expression to analysis overall survival, using publicly available gastric cancer microarray datasets (Kaplan-Meier Plotter). (G) Kaplan–Meier analysis of overall survival in patients with gastric cancer is achieved by examining the combination of *HOXC8* and *SPP1* mRNA levels (Kaplan-Meier Plotter). Other: low *HOXC8*/high *SPP1* and high *HOXC8*/low *SPP1*. (H) Comparison of OPN, pAKT, AKT, pERK, ERK, and β-actin levels between *HOXC8*-knockdown AZ521 cells and shluc control cells.

Discussion

In this study, the HOXC family was evaluated in patients with STAD, and *HOXC8* expression was upregulated in STAD tumor tissues and associated with worse overall survival. High levels of *HOXC8* mRNA expression also correlated with tumor stats, indicating that *HOXC8* plays a role in STAD progression. Although *HOXC8* reportedly mediate

cell viability in gastric cancer cell lines [36], the role of *HOXC8* in gastric cancer has not been fully evaluated. We revealed that *HOXC8* knockdown reduced the expression of OPN and phosphorylation of AKT/ERK in gastric cancer cells.

Accumulating evidence shows that deregulated *HOX* gene expression promotes malignant transformation in many cancers, including leukemias, breast, cervical, ovarian, prostate, colorectal, melanoma, and

squamous cell carcinoma. *HOX* genes reportedly have both oncogenic and tumor suppressing functions in cancer [25, 40, 41]. Furthermore, *HOXC8* mediates cancer progression in multiple cancers, including lung, cervical, and gastric cancer [13, 36, 42]. In our study, the *HOXC* family exhibited genetic alterations and co-occurrence in the same STAD patients. *HOXC8* was upregulated in STAD tissues and was associated with worse overall survival. High expression of *HOXC8* was associated with the tumor grades in STAD patients. Our data also revealed that *HOXC8* knockdown reduced the cell growth and colony formation ability in AZ521 and HR cells.

OPN is frequently observed in multiple human cancers, which contributes to tumor formation and progression. OPN expression is significantly upregulated in most gastric cancer patients at both the RNA and protein levels. Furthermore, OPN expression is significantly associated with a low apoptotic index, high proliferative index, low grade, high stage, lymph node and vascular invasion, and distal metastasis in the clinicopathology of gastric cancer patients [30, 43-47]. An earlier study showed OPN as a direct target of *HOXC8* in C57BL/6J mouse embryo fibroblast cells. OPN was downregulated in *HOXC8*-overexpressed cells [37]. Adwan *et al.* showed that *HOXC8* knockdown induced OPN expression in Suit2-007 cells [11]. In our study, *HOXC8* knockdown significantly inhibited the cell growth and colony formation, and decreased the expression of OPN in gastric cancer cells. The high *HOXC8*/high *OPN* expression group was associated with shorter overall survival compared to that of the low *HOXC8*/high *OPN* expression group among gastric cancer patients. These findings suggest that the *HOXC8*/OPN axis may play a role in gastric cancer progression.

An earlier study reported that OPN is responsible for modulating the AKT and MAPK pathways and cell proliferation *in vitro* [48]. In this study, we further evaluated the effect of *HOXC8* on the expression of OPN and the AKT/ERK pathway in gastric cancer cells. *HOXC8* knockdown significantly inhibited the expression of OPN and the phosphorylation of AKT/ERK in gastric cancer cell lines. We could not exclude the possibility that *HOXC8* directly or indirectly modulates OPN expression; however, this is the first study to evaluate alterations in *HOXC8* and OPN expression in gastric cancer cells.

Conclusions

After evaluating the genetic alteration of the HOXC family, we focused on *HOXC8* and its upregulation was associated with the tumor grade in patients with STAD. This study is the first to investigate the molecular mechanism by which *HOXC8* modulates cell growth, colony formation, and the OPN-related pathway of gastric cancer cells. We found that *HOXC8* expression correlates with poor overall survival in gastric cancer patients, indicating that *HOXC8* is an independent prognostic factor.

Abbreviations

HOX: homeobox; SPP1: secreted phosphoprotein 1; OPN: osteopontin; TCGA: The Cancer Genome Atlas; STAD: stomach adenocarcinoma; SMAD4: SMAD Family Member 4; PRDM4: PR Domain Zinc Finger Protein 4; GMNN: Geminin DNA Replication Inhibitor; ZFP90: ZFP90 Zinc Finger Protein; PBX2: PBX Homeobox 2; RBPMS: RNA Binding Protein, MRNA Processing Factor; PBX1: PBX Homeobox 1; PDE4DIP: Phosphodiesterase 4D Interacting Protein; PLA2G10: Phospholipase A2 Group X; ABL1: ABL Proto-Oncogene 1, Non-Receptor Tyrosine Kinase; C1orf109: Chromosome 1 Open Reading Frame 109; KRTAP12-1: Keratin Associated Protein 12-1; LHX2: LIM Homeobox 2; TEKT4: Tektin 4; LHX3: LIM Homeobox 3; TRIM42: Tripartite Motif Containing 42; FNTB: Farnesyltransferase, CAAX Box, Beta; BLZF1: Basic Leucine Zipper Nuclear Factor 1; CYSRT1: Cysteine Rich Tail 1; SMAD1: SMAD Family Member 1; KPRP: Keratinocyte Proline Rich Protein; ZRANB1: Zinc Finger RANBP2-Type Containing 1; FOXO1: Forkhead Box O1.

Availability of online data

In this study, we used publicly available online datasets from TCGA, TNMplot, TIMER and Kaplan–Meier analysis database.

Availability of data and materials

The datasets used and analysed during the current study available from the corresponding author on reasonable request.

Author contributions

Designed and wrote the manuscript: Y.-F. Y and C.-Y. T; performed experiments: Y.-C. L and Y.-F. Y; interpreted data: Y.-F. Y, J.-B. L and C.-Y. T.

Competing Interests

The authors have declared that no competing interest exists.

References

1. Torre LA, Siegel RL, Ward EM, Jemal A: Global Cancer Incidence and Mortality Rates and Trends--An Update. Cancer Epidemiol Biomarkers Prev 2016, 25:16-27.
2. Sitarz R, Skierucha M, Mielko J, Offerhaus GJA, Maciejewski R, Polkowski WP: Gastric cancer: epidemiology, prevention, classification, and treatment. Cancer Manag Res 2018, 10:239-248.
3. Collaborators GBDSC: The global, regional, and national burden of stomach cancer in 195 countries, 1990-2017: a systematic analysis for the Global Burden of Disease study 2017. Lancet Gastroenterol Hepatol 2020, 5:42-54.
4. Zhang XY, Zhang PY: Gastric cancer: somatic genetics as a guide to therapy. J Med Genet 2017, 54:305-312.
5. Koizumi W, Narahara H, Hara T, Takagane A, Akiya T, Takagi M, Miyashita K, Nishizaki T, Kobayashi O, Takiyama W et al: S-1 plus cisplatin versus S-1 alone for first-line treatment of advanced gastric cancer (SPIRITS trial): a phase III trial. Lancet Oncol 2008, 9:215-221.
6. Sasako M, Sakuramoto S, Katai H, Kinoshita T, Furukawa H, Yamaguchi T, Nashimoto A, Fujii M, Nakajima T, Ohashi Y: Five-year outcomes of a randomized phase III trial comparing adjuvant chemotherapy with S-1 versus surgery alone in stage II or III gastric cancer. J Clin Oncol 2011, 29:4387-4393.
7. Park JY, von Karsa L, Herrero R: Prevention strategies for gastric cancer: a global perspective. Clin Endosc 2014, 47:478-489.
8. Shah N, Sukumar S: The Hox genes and their roles in oncogenesis. Nat Rev Cancer 2010, 10:361-371.
9. Gehring WJ, Hiromi Y: Homeotic genes and the homeobox. Annu Rev Genet 1986, 20:147-173.
10. Brotto DB, Siena ADD, de B, II, Carvalho S, Muys BR, Goedert L, Cardoso C, Placa JR, Ramao A, Squire JA et al: Contributions of HOX genes to cancer hallmarks: Enrichment pathway analysis and review. Tumour Biol 2020, 42:1010428320918050.
11. Adwan H, Zhivkova-Galunska M, Georges R, Eyol E, Kleeff J, Giese NA, Friess H, Bergmann F, Berger MR: Expression of HOXC8 is inversely related to the progression and metastasis of pancreatic ductal adenocarcinoma. Br J Cancer 2011, 105:288-295.
12. Axlund SD, Lambert JR, Nordeen SK: HOXC8 inhibits androgen receptor signaling in human prostate cancer cells by inhibiting SRC-3 recruitment to direct androgen target genes. Mol Cancer Res 2010, 8:1643-1655.
13. Liu H, Zhang M, Xu S, Zhang J, Zou J, Yang C, Zhang Y, Gong C, Kai Y, Li Y: HOXC8 promotes proliferation and migration through transcriptional up-regulation of TGFbeta1 in non-small cell lung cancer. Oncogenesis 2018, 7:1.
14. de Bessa Garcia SA, Araujo M, Pereira T, Mouta J, Freitas R: HOX genes function in Breast Cancer development. Biochim Biophys Acta Rev Cancer 2020, 1873:188358.
15. Chen Z, Qin Z, Li L, Wo Q, Chen X: HOXA13, Negatively Regulated by miR-139-5p, Decreases the Sensitivity of Gastric Cancer to 5-Fluorouracil Possibly by Targeting ABCC4. Front Oncol 2021, 11:645979.
16. He Q, Huang W, Liu D, Zhang T, Wang Y, Ji X, Xie M, Sun M, Tian D, Liu M et al: Homeobox B5 promotes metastasis and poor prognosis in Hepatocellular Carcinoma, via FGFR4 and CXCL1 upregulation. Theranostics 2021, 11:5759-5777.
17. Ji M, Feng Q, He G, Yang L, Tang W, Lao X, Zhu D, Lin Q, Xu P, Wei Y et al: Silencing homeobox C6 inhibits colorectal cancer cell proliferation. Oncotarget 2016, 7:29216-29227.
18. Yin J, Guo Y: HOXD13 promotes the malignant progression of colon cancer by upregulating PTPRN2. Cancer Med 2021, 10:5524-5533.
19. Qiao C, Huang W, Chen J, Feng W, Zhang T, Wang Y, Liu D, Ji X, Xie M, Sun M et al: IGF1-mediated HOXA13 overexpression promotes colorectal cancer metastasis through upregulating ACLY and IGF1R. Cell Death Dis 2021, 12:564.
20. Ferrando AA, Armstrong SA, Neuberg DS, Sallan SE, Silverman LB, Korsmeyer SJ, Look AT: Gene expression signatures in MLL-rearranged T-lineage and B-precursor acute leukemias: dominance of HOX dysregulation. Blood 2003, 102:262-268.
21. Armstrong SA, Golub TR, Korsmeyer SJ: MLL-rearranged leukemias: insights from gene expression profiling. Semin Hematol 2003, 40:268-273.
22. Golub TR, Slonim DK, Tamayo P, Huard C, Gaasenbeek M, Mesirov JP, Coller H, Loh ML, Downing JR, Caligiuri MA et al: Molecular

23. classification of cancer: class discovery and class prediction by gene expression monitoring. Science 1999, 286:531-537.
23. Chen M, Qu Y, Yue P, Yan X: The Prognostic Value and Function of HOXB5 in Acute Myeloid Leukemia. Front Genet 2021, 12:678368.
24. Bhatlekar S, Viswanathan V, Fields JZ, Boman BM: Overexpression of HOXA4 and HOXA9 genes promotes self-renewal and contributes to colon cancer stem cell overpopulation. J Cell Physiol 2018, 233:727-735.
25. Miller GJ, Miller HL, van Bokhoven A, Lambert JR, Werahera PN, Schirripa O, Lucia MS, Nordeen SK: Aberrant HOXC expression accompanies the malignant phenotype in human prostate. Cancer Res 2003, 63:5879-5888.
26. Hamid AR, Hoogland AM, Smit F, Jannink S, van Rijt-van de Westerlo C, Jansen CF, van Leenders GJ, Verhaegh GW, Schalken JA: The role of HOXC6 in prostate cancer development. Prostate 2015, 75:1868-1876.
27. Shen WF, Krishnan K, Lawrence HJ, Largman C: The HOX homeodomain proteins block CBP histone acetyltransferase activity. Mol Cell Biol 2001, 21:7509-7522.
28. Bhatlekar S, Fields JZ, Boman BM: Role of HOX Genes in Stem Cell Differentiation and Cancer. Stem Cells Int 2018, 2018:3569493.
29. Chen SW, Zhang Q, Xu ZF, Wang HP, Shi Y, Xu F, Zhang WJ, Wang P, Li Y: HOXC6 promotes gastric cancer cell invasion by upregulating the expression of MMP9. Mol Med Rep 2016, 14:3261-3268.
30. Junnila S, Kokkola A, Mizuguchi T, Hirata K, Karjalainen-Lindsberg ML, Puolakkainen P, Monni O: Gene expression analysis identifies over-expression of CXCL1, SPARC, SPP1, and SULF1 in gastric cancer. Genes Chromosomes Cancer 2010, 49:28-39.
31. Wu CY, Wu MS, Chiang EP, Wu CC, Chen YJ, Chen CJ, Chi NH, Chen GH, Lin JT: Elevated plasma osteopontin associated with gastric cancer development, invasion and survival. Gut 2007, 56:782-789.
32. Senger DR, Wirth DF, Hynes RO: Transformed mammalian cells secrete specific proteins and phosphoproteins. Cell 1979, 16:885-893.
33. El-Tanani MK: Role of osteopontin in cellular signaling and metastatic phenotype. Frontiers in bioscience : a journal and virtual library 2008, 13:4276-4284.
34. Ghafouri-Fard S, Dashti S, Farsi M, Taheri M: HOX transcript antisense RNA: An oncogenic lncRNA in diverse malignancies. Exp Mol Pathol 2021, 118:104578.
35. Wang MQ, Yin QY, Chen YR, Zhu SL: Diagnostic and prognostic value of HOXC family members in gastric cancer. Future Oncol 2021, 17:4907-4923.
36. Gu H, Zhong Y, Liu J, Shen Q, Wei R, Zhu H, Zhang X, Xia X, Yao M, Ni M: The Role of miR-4256/HOXC8 Signaling Axis in the Gastric Cancer Progression: Evidence From lncRNA-miRNA-mRNA Network Analysis. Front Oncol 2021, 11:793678.
37. Lei H, Wang H, Juan AH, Ruddle FH: The identification of Hoxc8 target genes. Proc Natl Acad Sci U S A 2005, 102:2420-2424.
38. Zhang J, Yamada O, Kida S, Matsushita Y, Murase S, Hattori T, Kubohara Y, Kikuchi H, Oshima Y: Identification of brefelamide as a novel inhibitor of osteopontin that suppresses invasion of A549 lung cancer cells. Oncol Rep 2016, 36:2357-2364.
39. Yang YF, Chang YC, Jan YH, Yang CJ, Huang MS, Hsiao M: Squalene synthase promotes the invasion of lung cancer cells via the osteopontin/ERK pathway. Oncogenesis 2020, 9:78.
40. Flatte S: Calculations of wave propagation through statistical random media, with and without a waveguide. Opt Express 2002, 10:777-804.
41. Hung YC, Ueda M, Terai Y, Kumagai K, Ueki K, Kanda K, Yamaguchi H, Akise D, Ueki M: Homeobox gene expression and mutation in cervical carcinoma cells. Cancer Sci 2003, 94:437-441.
42. Huang Y, Chen L, Guo A: Upregulated expression of HOXC8 is associated with poor prognosis of cervical cancer. Oncol Lett 2018, 15:7291-7296.
43. Ue T, Yokozaki H, Kitadai Y, Yamamoto S, Yasui W, Ishikawa T, Tahara E: Co-expression of osteopontin and CD44v9 in gastric cancer. Int J Cancer 1998, 79:127-132.
44. Dai N, Bao Q, Lu A, Li J: Protein expression of osteopontin in tumor tissues is an independent prognostic indicator in gastric cancer. Oncology 2007, 72:89-96.
45. Higashiyama M, Ito T, Tanaka E, Shimada Y: Prognostic significance of osteopontin expression in human gastric carcinoma. Ann Surg Oncol 2007, 14:3419-3427.
46. Imano M, Satou T, Itoh T, Sakai K, Ishimaru E, Yasuda A, Peng YF, Shinkai M, Akai F, Yasuda T et al: Immunohistochemical expression of osteopontin in gastric cancer. J Gastrointest Surg 2009, 13:1577-1582.
47. Zhang X, Tsukamoto T, Mizoshita T, Ban H, Suzuki H, Toyoda T, Tatematsu M: Expression of osteopontin and CDX2: indications of phenotypes and prognosis in advanced gastric cancer. Oncol Rep 2009, 21:609-613.

48. Cao DX, Li ZJ, Jiang XO, Lum YL, Khin E, Lee NP, Wu GH, Luk JM: Osteopontin as potential biomarker and therapeutic target in gastric and liver cancers. World J Gastroenterol 2012, 18:3923-3930.