HIF2α is associated with poor prognosis and affects the expression levels of survivin and cyclin D1 in gastric carcinoma

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Abstract. Hypoxia-inducible factor-2α (HIF2α) is a major determinant factor of invasion and metastasis in various tumors. It has been reported that HIF2α is overexpressed in many tumors, including gastric cancer. However, the roles of HIF2α in the progression of gastric cancer are still not clear. In this study, we first examined the levels of HIF2α in gastric cancer by using immunohistochemistry, western blot and real-time PCR analysis. The results showed that HIF2α was highly expressed in gastric cancers compared to non-neoplastic mucosa and significantly correlated with histologic grade, TNM stages and peritoneal dissemination. MTT and colony formation assay revealed HIF2α overexpression induced high proliferation in BGC823 cells and HIF2α knockdown significantly inhibited proliferation in SGC7901 cells. Furthermore, we demonstrated that HIF2α could promote migration and invasion in gastric cancer cells. The results of western blot and RT-PCR analysis indicated that Survivin, Cyclin D1, MMP2 and MMP9 are upregulated with HIF2α overexpression. Finally, similar roles of HIF2α also *in vivo* were demonstrated. Taken together, the present study suggested that HIF-2α was involved in proliferation, metastasis and invasion of gastric cancer cells, with the induction of Survivin, Cyclin D1, MMP2 and MMP9 expression.

Introduction

Gastric cancer, a familiar malignant gastrointestinal tumor, is ranked as the second most common cancer related death in the world. Hypoxia microenvironment exists in many tumors due to structural and functional abnormality of vessels and increased oxygen consumption caused by rapid proliferation of tumor cells. Under these circumstances, tumor cells can survive under hypoxic conditions by expressing proteins such as angiogenic factors, glycolytic enzymes and stress proteins which promote their survival (1-4). Many of the hypoxia adaptations are mediated by the activation of specific genes through hypoxia-inducible factor (HIF-1 and HIF-2). These heterodimeric transcription factors consist of an oxygen-sensitive α subunit (HIF-1α or HIF-2α) that forms an active complex with HIF-β. In normoxia, the HIFα subunits are degraded via the proteasomal pathway (5-9). Increasing evidence has demonstrated that HIF1α and HIF2α are important factors in prompting aggressiveness in many cancers, including gastric cancer, breast cancer, colon cancer, rectal cancer, head and neck cancer and neuroblastoma (10-17). During the process of tumor development, HIF2α implements different functions, such as angiogenesis, proliferation, and tumor stem cells, through mediated the target genes (8,18).

In this study, we investigate the relationship between the expression of HIF2α and the clinical characteristics of patients with gastric cancer. Next, we demonstrated HIF2α might influence the proliferative, migrate, invasive abilities of gastric cancer cells both *in vitro* and *in vivo*. HIF2α expression induces high expression of Survivin, Cyclin D1, MMP2 and MMP9 in gastric cancer cells.

Materials and methods

Patient information and specimens. Paraffin specimens (n=127) were obtained from patients with gastric carcinoma who underwent surgery or biopsy at the First Affiliated Hospital of China Medical University between 2007 and 2011. Follow-up data were obtained from review of the patients medical record. The TNM staging system of the Union for International Cancer Control (UICC) was used to classify specimens as stages I (n=18), II (n=26), III (n=20), and IV (n=63). None of the patients had received radiotherapy or chemotherapy before surgical resection or biopsy. Twenty cases (included in the 127 cases) of tumor and adjacent non-neoplastic mucosa (NNM) were quickly frozen at -70˚C until protein and RNA extraction. The study was approved by the Ethics Committee of China Medical Univesity. The enrolled patients agreed in writing their tissue samples could be used for scienctific research, prior to surgery or biopsy.

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Immunohistochemistry. The tissues were fixed with neutral formalin and embedded in paraffin. Five-micrometer thick continuous sections were prepared for immunohistochemical staining by the streptavidin-peroxidase (S-P) method. The samples were incubated with HIF2α polyclonal antibody (1:100, Abcom, USA), at 4˚C overnight. The following day after combination with secondary antibody labeled biotin, the primary antibodies were detected by the appropriate labeled streptavidin-peroxidase. Immunolabeled sections were visualized with 3, 3'-diaminobenzidine, and counterstained with hematoxylin. The negative control was performed with PBS instead of primary antibody to exclude the non-specific binding of the secondary antibody.

Evaluation of immunostaining. All of the stained sections were assessed by two pathologists (X.H. Li and Y. Zhao, China Medical University). Five views were randomly examined per slide, and 100 cells were observed per view at x400 magnification. HIF2α expression was classified into five groups depending on the percentage of positively staining cells: 0 (0 score), 1-25% (1 score), 26-50% (2 score), 51-75% (3 score), and >75% (4 score). Immunohistochemical staining intensity was graded as: no staining (0 score), light staining (1 score), middle staining (2 score), heavy staining (3 score). The product of the staining intensity score and positive HIF2α percentage score was considered as the final score for each sample. Cases with total score ≤4 were considered 'negative expression' and >4 score was 'positive expression'.

Cell culture. The normal human gastric mucosa epithelial cell line GES1 and the human adenocarcinoma cell line MKN45 were obtained from the Cell Bank of Chinese Academy of Science (Shanghai, China). BGC823 and SGC7901 were obtained from the Academy of Military Medical Science (Beijing, China). Cell lines were cultured in RPMI-1640 medium (Gibco, Inc., Grand Island, NY, USA) containing 10% fetal bovine serum and penicillin-streptomycin at 37˚C in a humidified atmosphere with 5% CO_2.

Plasmid, heterogeneous nuclear RNA (hnRNA), and transfection. HIF2α target gene was constructed into pcDNA3.1-flag (Promega, USA) carrier by molecular cloning technique. The hnRNA expression vector for HIF2α was constructed containing a HIF2α-specific targeting sequence (shRNA HIF2α-#1: 5'-GACAAGGTCTGCAAAGGGT-3'; shRNA HIF2α-#2: 5'-CGACCTGAAGATTGAAGTGAC-3') and negative control hnRNA (shRNA-NC: 5'-TTCTCCGAACGTGTCACGT-3). For transient transfection, cells were transfected with Lipofectamine 2000 according to the manufacturer's instructions (Invitrogen, Carlsbad, CA, USA).

Western blot analysis. Total protein was extracted with RIPA lysis buffer. Protein lysates (40 μg) were separated on a 10% SDS-polyacrylamide gel and transferred to the polyvinylidene fluoride (PVDF) membranes. After blocking, the blots were incubated with primary antibodies against HIF2α (Abcam, Cambridge, MA, USA), Cyclin D1, Survivin, MMP2, MMP9 (Cell Signaling, Boston, MA, USA), GAPDH (Santa Cruz Biotechnology, Santa Cruz, CA, USA) at 4˚C overnight. The blots were then incubated with corresponding secondary antibodies, respectively, at 37˚C for 2 h. The blots were detected with ECL (Santa Cruz) and the Bio-Imaging System (UVP, Upland, CA, USA) was used to measure the gray intensity of the blots. The experiments were repeated three times independently.

Reverse transcription (RT)-polymerase chain reaction (PCR). Reverse transcription (RT)-polymerase chain reaction (PCR) Total RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's instructions. RT-PCR was performed with the AMV Ver3.0 kit (Takara, Shiga, Japan). Thirty cycles were used for *HIF2α, Survivin, Cyclin D1, MMP2, MMP9* and 25 cycles for *GAPDH*, respectively. The PCR products were electrophoresed in a 2% agarose gel containing 0.1 mg/ml of ethidium bromide. Then they were visualized and analyzed by The Bio-Imaging System (UVP). To provide a value for the transcriptional level of each gene, a gray scale intensity value was determined for each target band and normalized to *GAPDH*. Each experiment was repeated three times independently. The primer sequences were: for *HIF2α*, 5'-ACCTGAAGATTGAAGTGATTGAG-3' and 5'-GTGGCTGGAAGATGTTTGTC-3'; for *Cyclin D1*, 5'-TGGAGCCCGTGAAAAAGAGC-3' and 5'-TCTCCTTCATCTTAGAGGCCAC-3'; for *Survivin*, 5'-GGTCTCCTCTGACTTCAACA-3' and 5'-AGCCAAATTCGTTGTCATAC-3'; for *MMP2*, 5'-ATGACATCAAGGGCATTCAGGAG-3' and 5'-TCTGAGCGATGCCATCAAATACA-3'; for *MMP9*, 5'-TCCCAGACCTGGGCAGATTC-3' and 5'-GCAAAGGCGTCGTCAATCAC-3'; for *GAPDH*, 5'-TGGTGAAGGTCGGTGTGAAC-3' and 5'-CCATGTAGTTGAGGTCAATGAAGG-3'.

MTT assay. The single cell suspension (200 μl) diluted with RPMI-1640 containing 10% fetal bovine was inoculated into 96-well plates, followed by cultivation for 24 h at 37˚C, 5% CO_2 in saturated humidity conditions. The cells were incubated with tetrazolium dye MTT at 37˚C, then dissolved in DMSO (200 μl), and determined by enzyme linked immune meter (Bio-Rad, USA).

Colony formation experiment. Cell suspension (5 ml) containing 95% of single cells was inoculated in culture dishes after they were multiple proportions diluted according to cell proliferative ability. Cells grew at the condition of 37˚C and 5% CO_2 for 2-3 weeks, fresh nutrient solution was replaced based on the medium pH change. Finally the formatted cell colonies were visualized with Giemsa (Leagene, Beijing, China).

Transwell assay. Matrigel (BD Biosciences) and Transwell inserts of 8.0-mm pore size (Corning, Inc., NY, USA) were used for invasion assays. Briefly, Matrigel diluted with serum-free medium at a ratio of 1:3 (100 ml) was added to the upper chamber, and 100 μl of cell suspension ($2x10^4$ cells/ml) was added after the gel formed. Six hundred microliters of medium containing 10% FBS was added to the lower chamber as the chemoattractant. For migration assay, cell suspension was added into the chamber directly without gel formation. After incubation for 48 h, the filters were fixed with 100% methanol for 15 min and then subjected to trypan blue staining (Sunshine, Nanjing, China). The cells that invaded and moved

Table I. HIF2α expression and the clinicopathological factors in patients with gastric cancer.

Clinicopathological factors	n	Negative expression (%)	Positive expression (%)	P-value
Gender				
Male	87	42 (48.3)	45 (51.7)	0.384
Female	40	16 (40)	24 (60)	
Age (years)				
≤60	79	38 (48.1)	41 (51.9)	0.48
>60	48	20 (41.7)	28 (58.3)	
Size of tumor (cm)				
≤5	79	39 (49.4)	40 (50.6)	0.283
>5	48	19 (39.6)	29 (60.4)	
Location of tumor				
Widely	14	9 (64.3)	5 (35.7)	0.141
Up 1/3	16	5 (31.2)	11 (68.8)	
Middle 1/3	21	13 (59.1)	9 (40.9)	
Down 1/3	75	31 (41.3)	44 (58.7)	
Differentiation				
Well to moderately	49	30 (61.2)	19 (38.8)	0.005
Poor	78	28 (35.9)	50 (64.1)	
Lymph node metastasis				
No	52	24 (46.2)	28 (53.8)	0.927
Yes	75	34 (45.3)	41 (54.7)	
pTNM staging				
I	18	12 (66.7)	6 (33.3)	0.016
II	26	15 (57.7)	11 (42.3)	
III	20	11 (55.0)	9 (45.0)	
IV	63	20 (31.7)	43 (68.3)	
Peritoneum metastasis				
No	106	54 (50.9)	52 (49.1)	0.007
Yes	21	4 (19.0)	17 (81.0)	

onto the lower surface of the filter membrane were counted in 10 random high power fields (x400) by an inverted microscope. The experiment was repeated thrice and the data are shown as mean + standard deviation (SD).

Nude mice bearing tumors. Thirty-six female BALB/c mice were randomly separated into three groups, one group was used as control with no-load BGC823 cells, the other two groups had tumors with HIF2α in BGC823 cells. BGC823 cells were prepared at a concentration of $1x10^7$ cells/ml in PBS, and intravenously injected into nude mice. The mice were fed normally for eight weeks then euthanized, dissected, and lung tissues harvested and photographed. Fixed with 4% paraformaldehyde, seven uniform plane sections of mouse lung tissues were prepared for H&E staining. All of the stained sections were observed for tumor cell cloning and counted. All experiments with animals were performed according to the guidelines of China Medical University Ethics Committee.

Statistical analysis. SPSS version 13.0 for Windows was used for all analyses. The Pearson Chi-square test was used to examine the correlation between the expression of HIF2α and clinicopathological factors. The Student's t-test was used to compare data from the densitometry analysis of western blot and RT-PCR analysis. The Kaplan-Meier method was used to estimate the probability of patient survival. Differences were considered significant when $P<0.05$.

Results

HIF2α expression was associated with poor differentiation, high pTNM stage, peritoneum metastasis, and poor prognosis in GC. In twenty cases of normal gastric tissues, HIF2α was not expressed in normal mucosa and the expression levels were scored as (-), as an internal control (Fig. 1A). However, in the 127 GC specimens, HIF2α expression was negative in 58 samples (45.7%) (Fig. 1B) and positive in 69 samples (54.3%) (Fig. 1C and D). Then we analyzed the relationship between HIF2α expression and clinicopathological factors in 127 GC samples and found that the positive expression of HIF2α was positively correlated with poor differentiation, high pathologic TNM (pTNM) stage, and peritoneum metastasis of gastric cancer (P=0.005; P=0.016; P=0.007, Table I). In the 78 poorly differentiated samples, HIF2α was positively expressed in

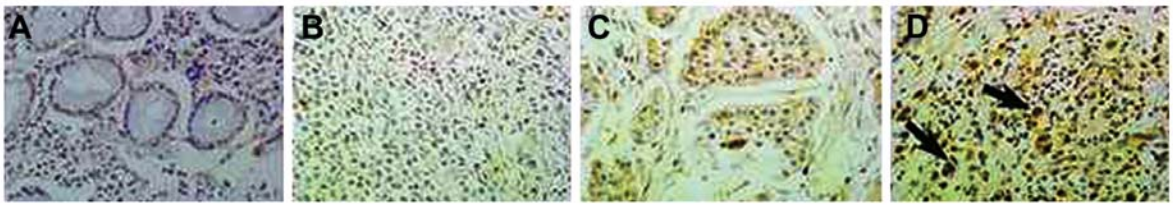

Figure 1. Immunostaining of HIF2α in GC. The same visual field of serial sections showed that HIF2α was not expressed in normal mucosa scored as the 0 score (A), HIF2α expression was the negative ≤4 score (B) and HIF2α expression was the positive >4 score (C and D), as indicated in the Materials and methods. Original magnification, x400; scale bar, 20 μm.

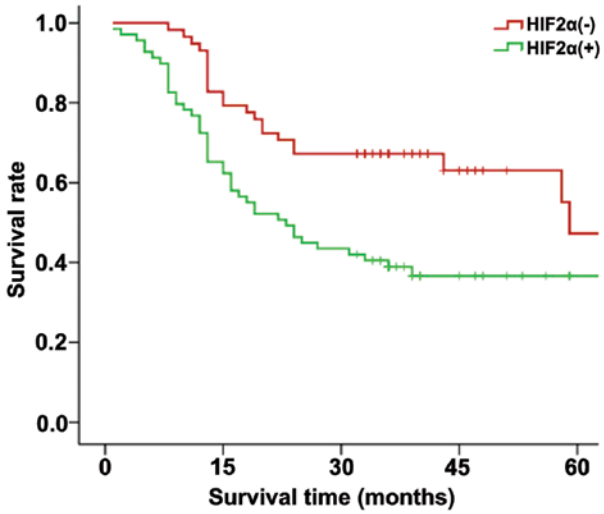

Figure 2. Positive HIF2α expression was correlated with poor prognosis in patients with gastric cancer (P=0.009).

50 samples (64.1%). In the 63 cases with pTNM stages IV, HIF2α was positive in 43 samples (68.3%). In the 21 cases with peritoneum metastasis, positive expression of HIF2α was observed in 17 samples (81.0%). In addition, statistical analysis showed that positive expression of HIF2α was not correlated with age, gender, size of tumor, location of tumor and lymph node metastasis (P>0.05, Table I). Moreover, in the 127 GC cases, patients with HIF2α positive expression had a significantly lower survival time than patients with negative expression of HIF2α (P=0.009, Fig. 2). The results of western blot and real-time PCR analysis showed that HIF2α protein and mRNA in gastric cancer tissues were significantly higher compared to those in the corresponding non-tumor gastric tissues (P<0.05, Fig. 3). We also found that the HIF2α protein and mRNA levels in MKN45, BGC823 and SGC7901 were higher than that in GES1 (P<0.05). HIF2α expression in SGC7901 was higher than that in SGC823 and MKN45 (Fig. 4).

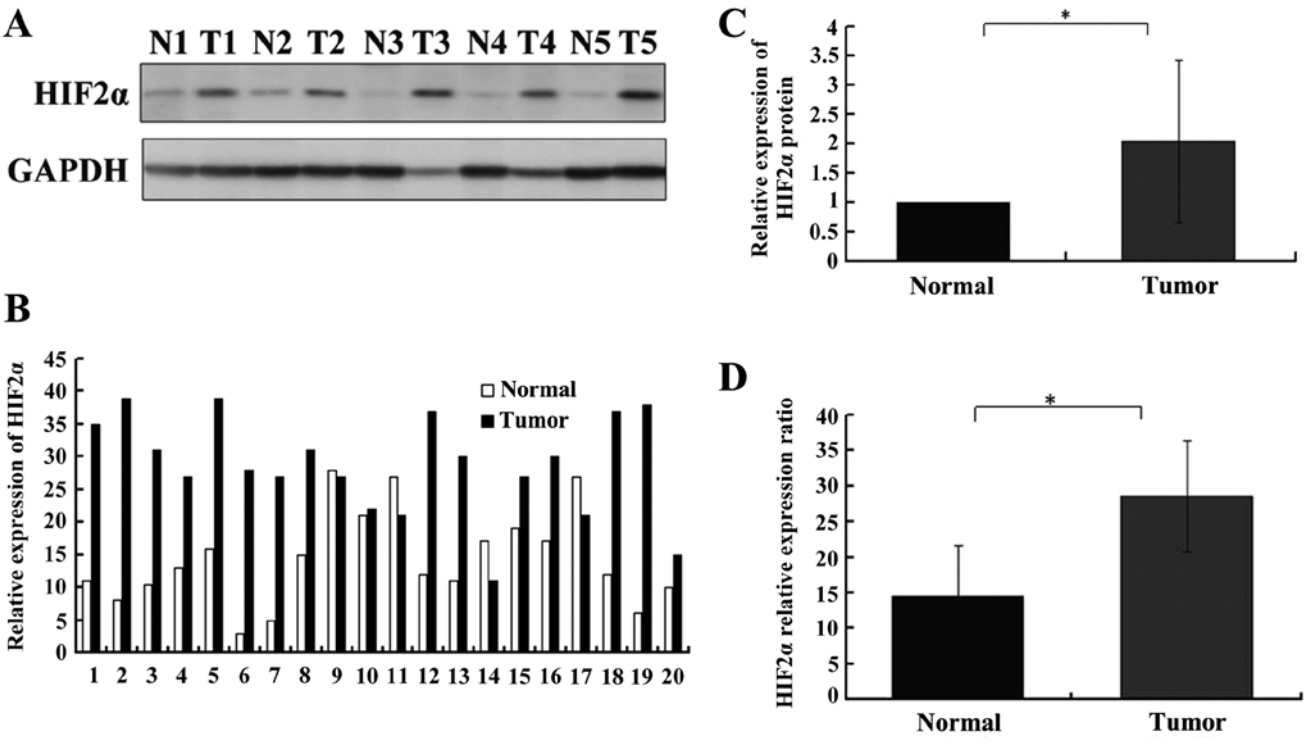

Figure 3. Expression of HIF2α in gastric cancer tissues. The expression of HIF2α protein and mRNA in the gastric cancer tissues (T1-T5) were significantly higher than corresponding non-tumor tissues (N1-N5) (P<0.05) (A and C for protein), (B and D for mRNA). Normal, non-tumor tissues; tumor, gastric cancer tissues.

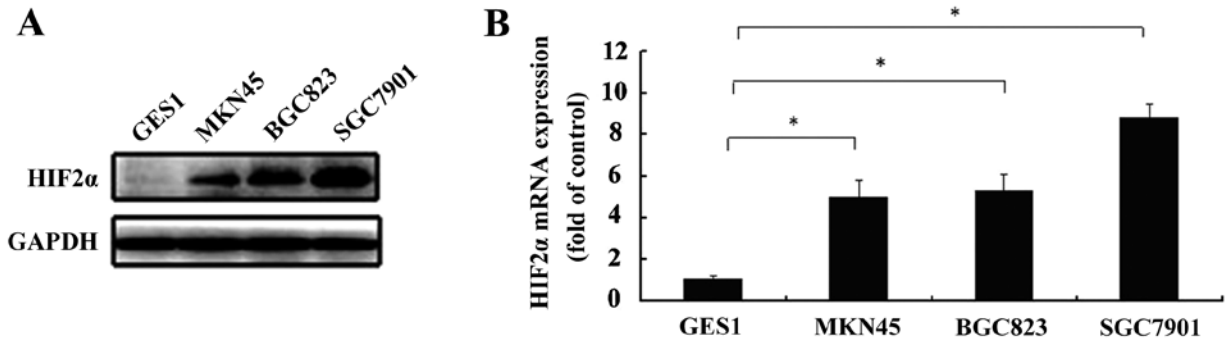

Figure 4. Expression of HIF2α in cell lines. HIF2α protein and mRNA expression in in MKN45, BGC823 and SGC7901 were all higher than in GES1 (P<0.05). HIF2α expression in SGC7901 was higher than in SGC823 and MKN45.

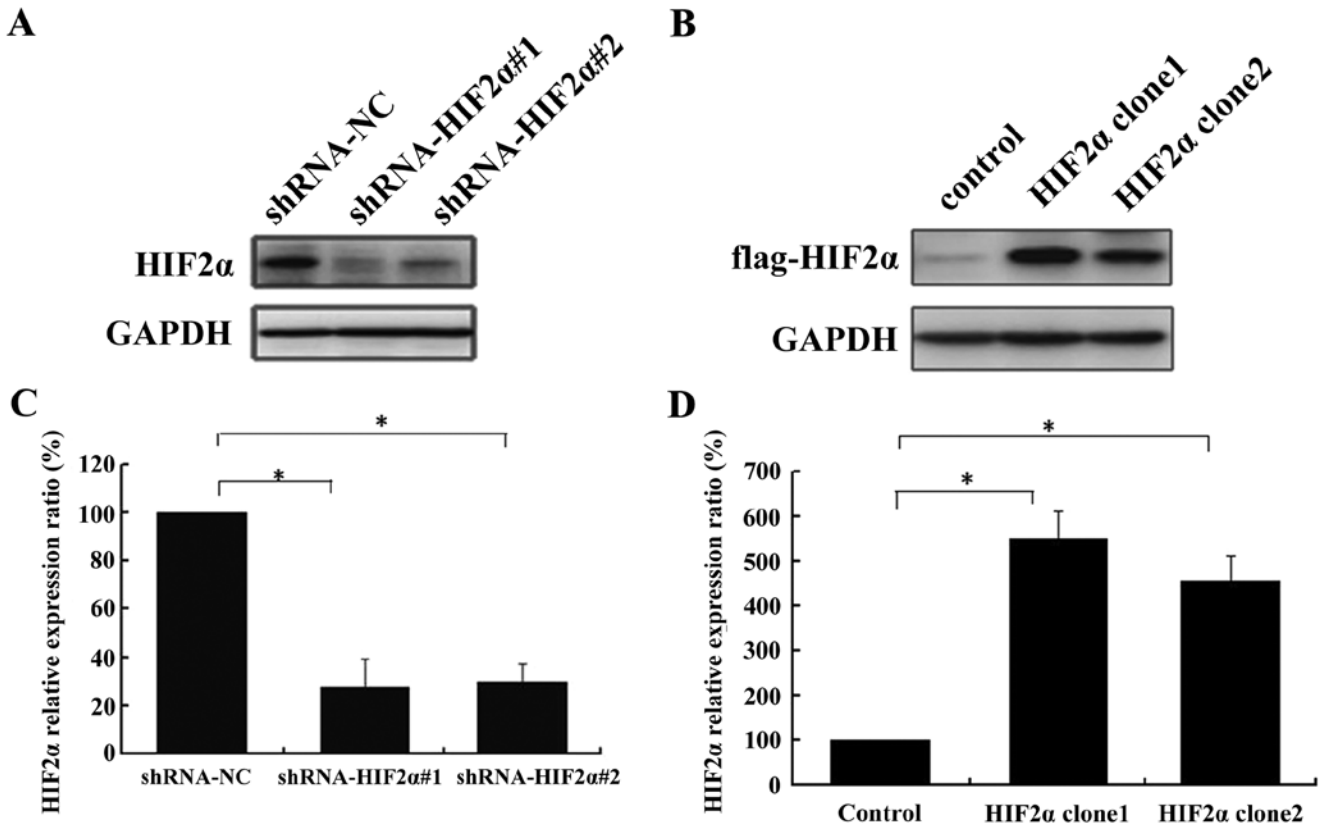

Figure 5. HIF2α expression promotes gastric cancer cell proliferation. HIF2α shRNA transfection resulted in a significant decrease of HIF2α expression in SGC7901 cells (P<0.05) (A and C). HIF2α pcDNA3-flag plasmid transfection resulted in a significant increase of HIF2α expression in BGC823 cells (P<0.05) (B and D).

HIF2α expression promotes gastric cancer cell proliferation. To provide further evidence that HIF2α expression was associated with gastric cancer cell biological characteristics, HIF2α shRNA was introduced in SGC7901 cells, and BGC823 cells were transfected with HIF2α pcDNA3-flag plasmid. Real-time PCR and western blotting were used to test the effect of HIF2α shRNA and HIF2α plasmid. The results showed that HIF2α expression decreased along with increasing shRNA concentrations in SGC7901 cells and HIF2α expression increased in BGC823 cells (Fig. 5).

To evaluate the effect of HIF2α in gastric cancer cell proliferation, MTT and colony formation assay was performed in HIF2α knockdown SGC7901 cells and HIF2α overexpressing BGC823 cells, the two kinds of cells used as negative control had no transfection. The proliferation of HIF2α knockdown SGC7901 cells was lower than the control ones. HIF2α overexpressing BGC823 cells showed higher proliferative rate than the untransfected ones (Fig. 6). Furthermore, the colony formation assay also confirmed similar results to the MTT assay (Fig. 7). The results indicated HIF2α expression was positively associated with gastric cancer cell proliferation.

HIF2α expression was associated with survivin and cyclin D1 expression in gastric cancer cells. To further explore whether HIF2α had an effect on the transcriptional activity of cyclin D1 and survivin, we examined the protein expression and mRNA

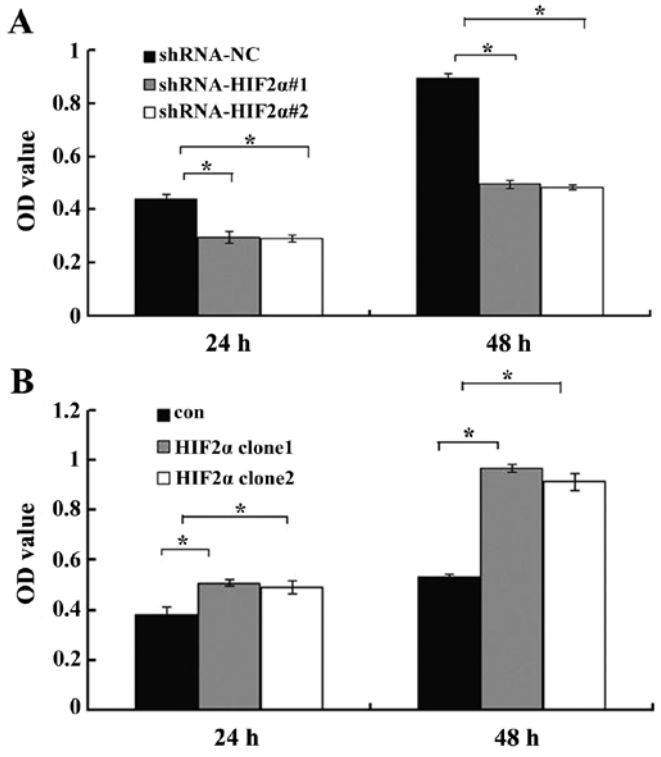

Figure 6. HIF2α expression promoted gastric cancer cell proliferation in MTT assay. shRNA-HIF2α SGC7901 cells proliferated slower than control (P<0.05) (A). HIF2α overexpressing BGC823 cells proliferated more quickly than control (P<0.05) (B).

levels of cyclin D1 and survivin in HIF2α shRNA-transfected SGC7901 cells and HIF2α pcDNA3-flag-transfected BGC823 cells. The results showed that the protein and the mRNA levels of cyclin D1 and survivin in HIF2α shRNA-transfected SGC7901 cells were significantly lower than the control ones (Fig. 8A and C), and in HIF2α pcDNA3-flag-transfected BGC823 cells were significantly higher than the control ones (Fig. 8B and D). Therefore, HIF2α expression led to upregulation of cyclin D1 and survivin protein expression and mRNA.

HIF2α expression promotes migration and invasive abilities in gastric cancer cells by regulating MMP2 and MMP9 expression. To explore the relationship between HIF2α and the migration and invasive abilities of gastric cancer cells, Matrigel invasion and migration assay was performed in HIF2α knockdown SGC7901 cells and HIF2α overexpression in BGC823 cells, using two cell lines as negative control without transfection. The results showed that the migration and invasive ability of the SGC7901 cells significantly enhanced with increasing concentrations of HIF2α shRNA compared to control (Fig. 9A and C), and the BGC823 cells transfected with HIF2α pcDNA3-flag showed lower mobility than the cells without transfection (P<0.05) (Fig. 9B and D). Furthermore, we examined the protein expression and mRNA levels of MMP2 and MMP9 in HIF2α shRNA-transfected SGC7901 cells and HIF2α pcDNA3-flag-transfected BGC823 cells. The results showed that the protein and the mRNA levels of MMP2 and MMP9 in HIF2α SGC7901 cells were significantly lower than the control ones (Fig. 10A and C), and in BGC823 cells were significantly higher than the control ones (Fig. 10B and D). It can be concluded that HIF2α expression led to upregulation of MMP2 and MMP9 protein expression and mRNA.

HIF2α expression promotes tumor metastasis in vivo. To explore the effect of HIF2α on gastric cancer metastasis *in vivo*, we performed intravenous injection into BALB/c nude mice with $1x10^7$ HIF2α stable expression BGC823 cells and no-load BGC823 cells as negative control, and sacrificed the mice eight weeks later. HIF2α BGC823 cells significantly improved the pulmonary metastasis of gastric cancer cells compared with no-load (Fig. 11A). The results of transferred tumor cell cloning count of HIF2α showed that BGC823

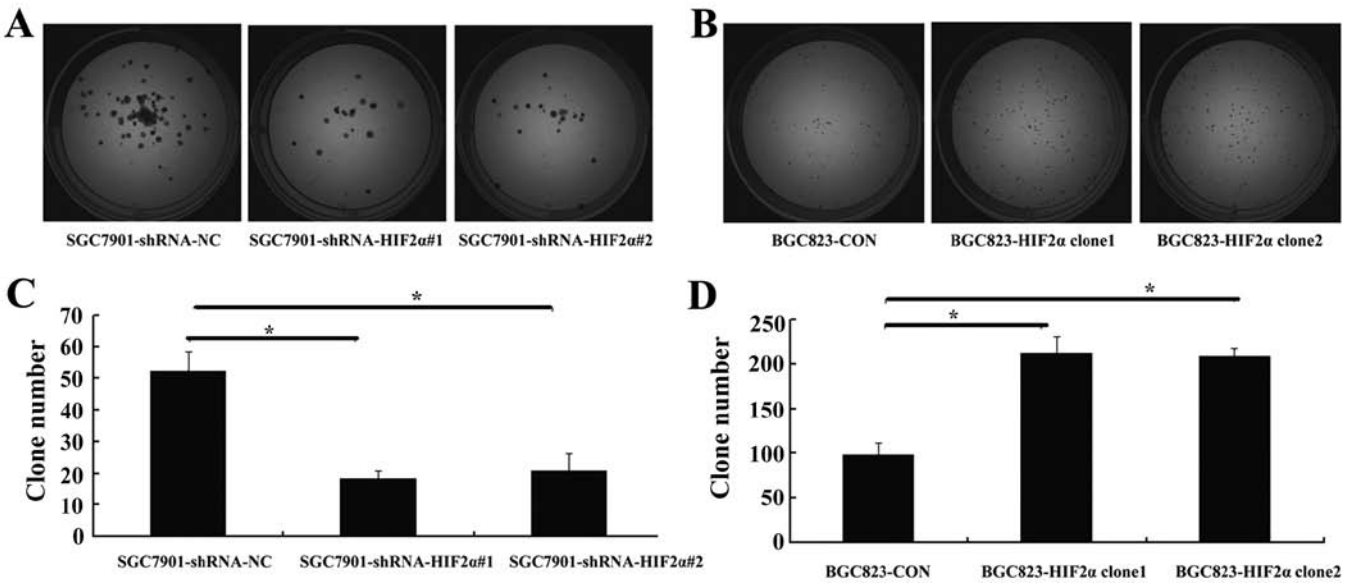

Figure 7. HIF2α expression promotes gastric cancer cell proliferation in colony formation assay. shRNA-HIF2α SGC7901 cells proliferated slower than control (P<0.05) (A and C), HIF2α overexpressing BGC823 cells proliferated more quickly than control (P<0.05) (B and D).

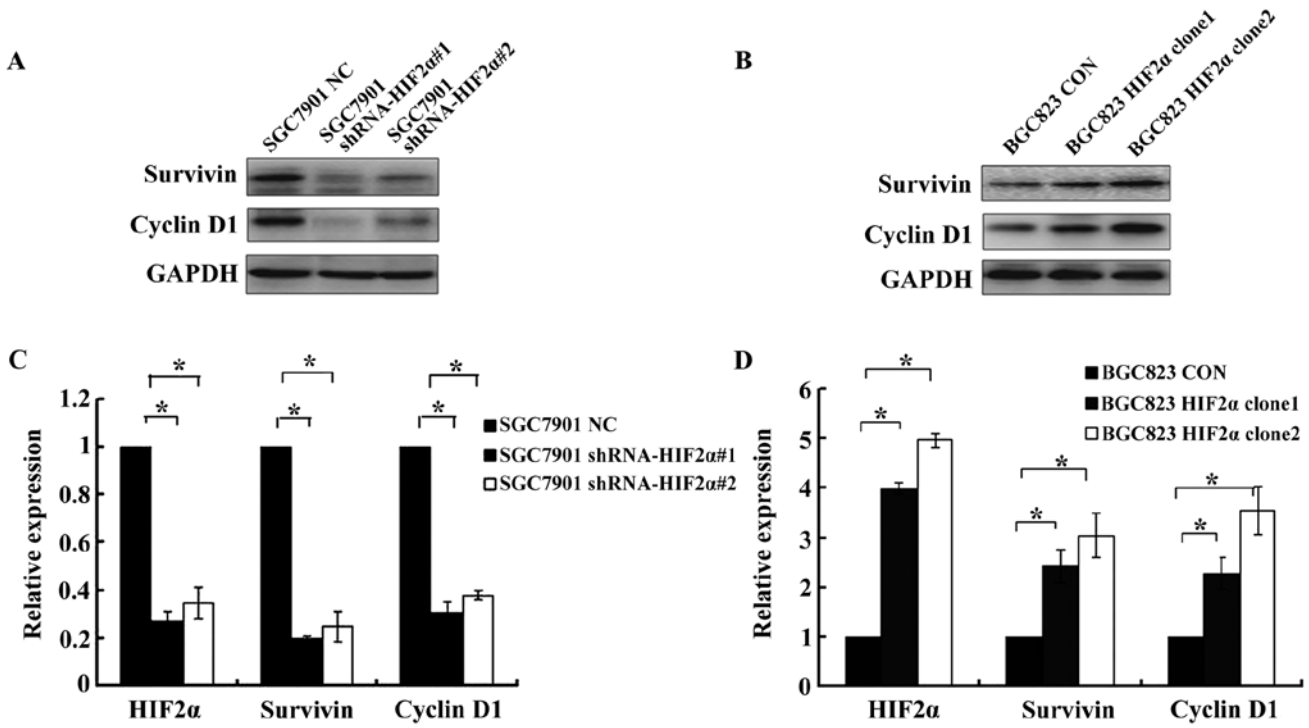

Figure 8. HIF2α expression was associated with cyclin D1 and survivin expression in gastric cancer cell. The protein expression and mRNA of cyclin D1 and survivin in shRNA-HIF2α SGC7901 cells were significantly lower than control SGC7901 (P<0.05) (A and C), and in HIF2α pcDNA3-flag-transfected BGC823 cells were significantly higher than control BGC823 (P<0.05) (B and D).

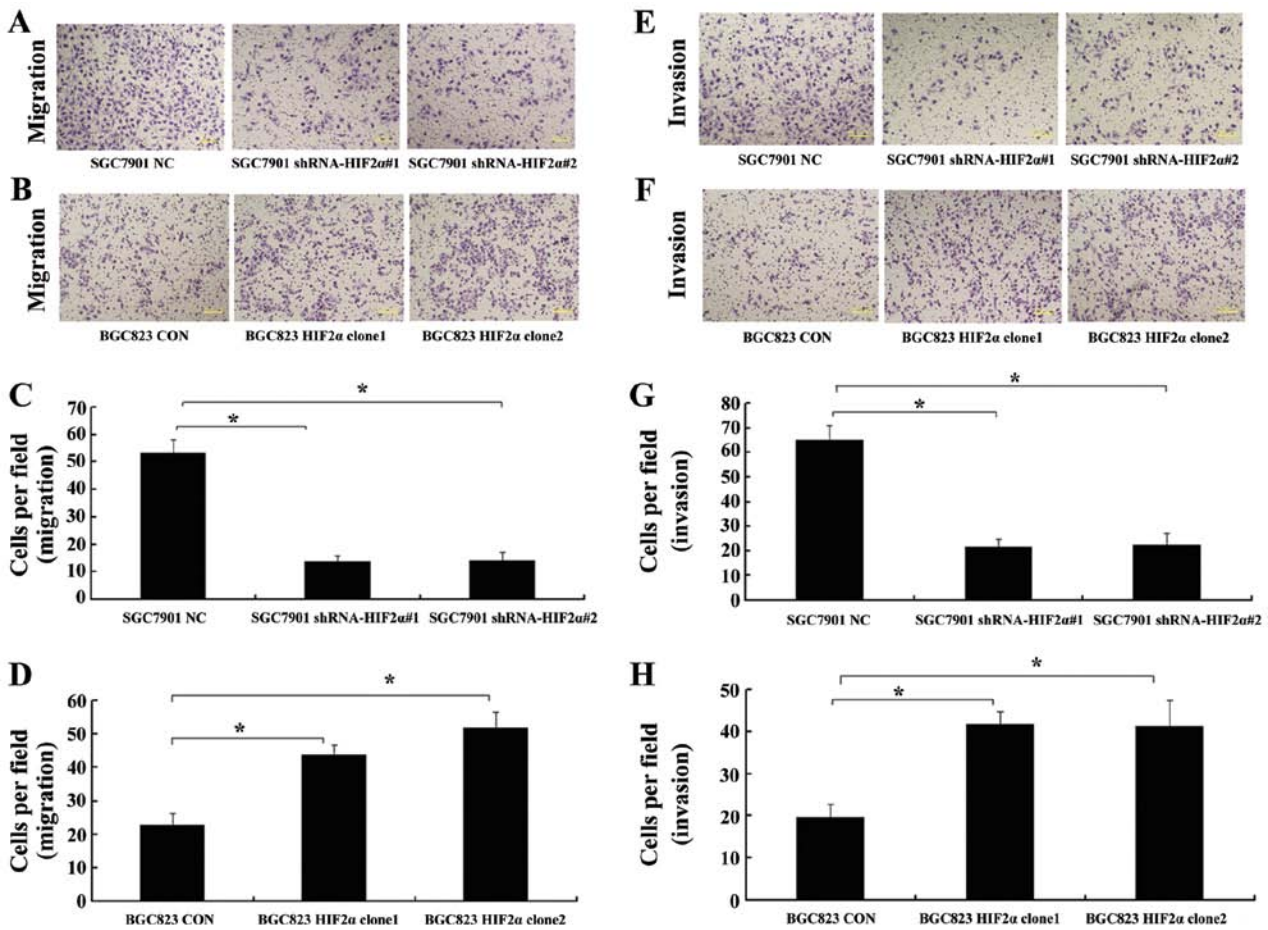

Figure 9. HIF2α expression promotes migration and invasive abilities in gastric cancer cells. The migration and invasive ability of the SGC7901 cells was significantly enhanced with increasing concentrations of HIF2α shRNA compared to control (P<0.05) (A, C, E and G), and the BGC823 cells transfected with HIF2α pcDNA3-flag had fewer migrated and invasive cells than the cells without transfection (P<0.05) (B, D, F and H).

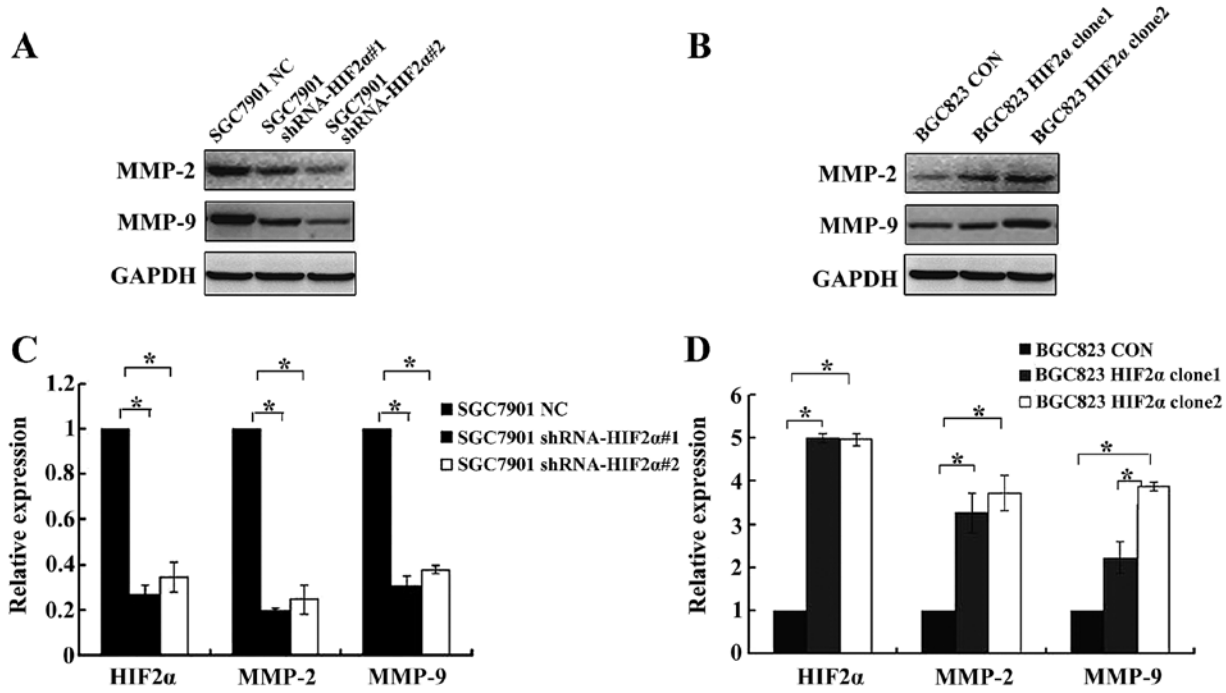

Figure 10. HIF2α expression is associated with MMP2 and MMP9 expression in gastric cancer cells. The protein expression and mRNA of MMP2 and MMP9 in shRNA-HIF2α SGC7901 cells were significantly lower than control (P<0.05) (A and C), and in HIF2α pcDNA3-flag-transfected BGC823 cells were significantly higher than control (P<0.05) (B and D).

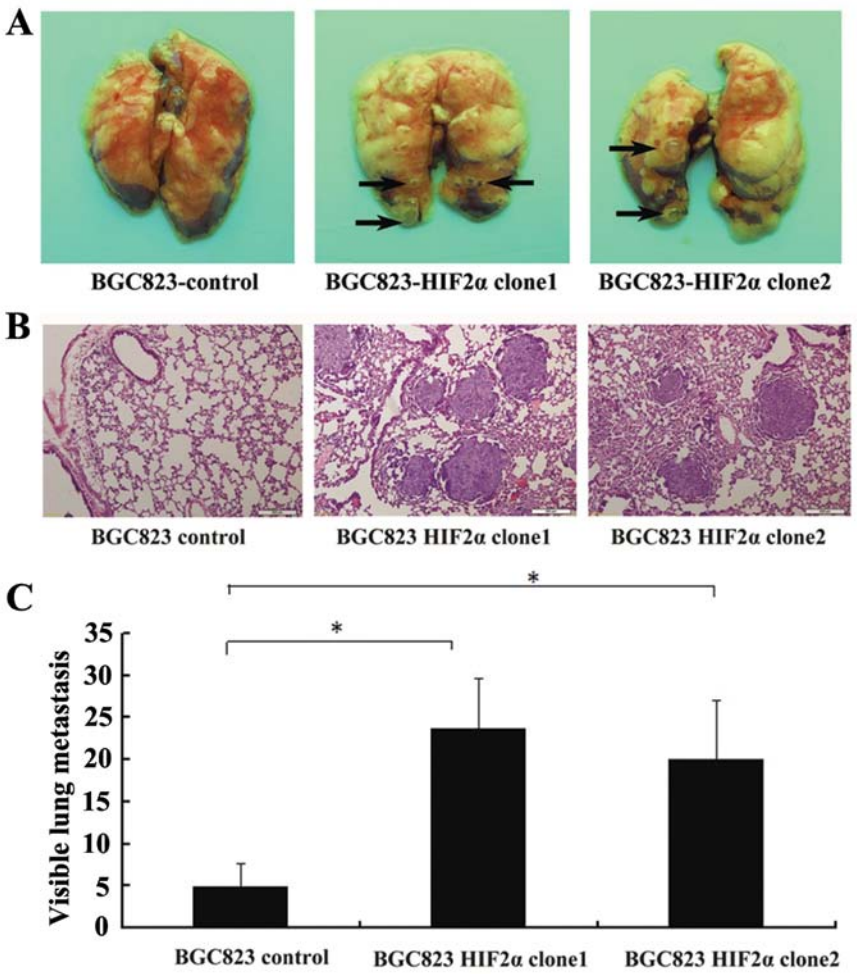

Figure 11. HIF2α expression promotes tumor metastasis *in vivo*. HIF2α in BGC823 cells significantly improved the pulmonary metastasis of gastric cancer cells compared with no-load (A). The results of transferred tumor cell cloning count of seven plane slice, HIF2α BGC823 cell cloning was significantly higher than the control group (P<0.05) (B and C).

cell cloning was significantly higher than the control group (P<0.05, Fig. 11B and C).

Discussion

Hypoxic cells are found in solid tumors, and evidence indicates that tumor cells adapt to hypoxia by increasing synthesis of the HIF proteins (19-21). Metastasis, characterized by the seeding and growth of satellite lesions in other organs, is commonly thought to be the final stage of cancer (22,23). The exact mechanisms of metastasis are not well defined, but the propensity to metastasize is suggested to be related to HIF (24,25). In some tumors, a positive association is observed between HIF-1α and tumor stage. In renal cancer and neuroblastoma, however, HIF-2α but not HIF-1α promotes an aggressive phenotype (15,16). In this study, we presented the first evidence that HIF2α are highly expressed in gastric cancer, and significantly correlate with poor differentiation, high pTNM stage, peritoneum metastasis in gastric cancer samples. This result was consistent with previous reports on HIF-1α and HIF-2α expression in gastric cancer (26).

We hypothesized that HIF-2α was involved in the proliferation of gastric cancer. To test this hypothesis, MTT and colony formation assay was performed in HIF2α knockdown SGC7901 cells and HIF2α overexpressing BGC823 cells, the gastric cells grew quickly with HIF-2α expression. The results indicated HIF2α expression may increase the proliferation of the gastric cancer BGC823 cells and decrease the SGC7901 cells that are HIF2α-dependent.

The proliferative mechanism mediated by HIF2α is poorly understood. Some data have demonstrated that the target gene mechanisms regulate cell proliferation (27,28). In an early primary embryonic stage, HIF2α plays a specific role in the transcription regulation of VEGF receptor-2 (Flk 1), and is the essential factor in the generation of the cardiovascular system (29). Using a technique called RNA interference silence HIF2α, CITED2, WISP2 and IGFBP3 are also found as specific target genes by HIF2α regulation (30). Oct 4 belongs to transcription factors which play an important role in stem cell self-renewal, HIF2α can induce Oct 4 expression by specific binding to promoter regions (31). In addition, the cell cycle protein (cyclin D1), transforming growth factor α (TGFα) expression is subject to HIF2α control and subsequently promotes the proliferation of tumor in RCC cell line (30). In the Kras induced human non-small cell lung cancer cell line (NSCLC), the suppressor gene expression of Scgb3a1 decreases due to HIF2α knockout, which eventually leads to the development of tumor, proving Scgb3a1 as one of the direct HIF2α target genes (32). The related genes existing in the process of skeletal muscle fiber-type switching belong to HIF2α specific target genes, for instance, the mRNA level of MyoHCI, Myoglobin, Calmodulin2 and Troponin I were upregulated by HIF2α, however, MyoHCIIb was downregulated by HIF2α (33).

We examined whether HIF2α accelerates proliferation in gastric cancer cells through induction of target gene activation, and subsequent Survivin and Cyclin D1 upregulation along with increase in HIF2α expression. Survivin and Cyclin D1 play an important part in the development process of many kinds of tumor, and HIF2α can implement positive regulation of Survivin and Cyclin D1 expression. This partly explains the mechanism by which Survivin and Cyclin D1 contribute to HIF-2α-induced proliferation in gastric cancer.

Metastasis leads to death in cancer patient, and invasion seems to be the most critical process in metastasis. We advanced this hypothesis asking whether HIF2α mediated migration and invasion through induction of certain gene activation in gastric cancer cells. Cell culture in Matrigel resulted in a significant increase in HIF2α-mediated migration and invasion in BGC823 cells and pretreatment of SGC7901 cells with hnRNA effectively inhibited the HIF2α-mediated increase in migration and invasion. These data suggest that HIF2α may participate in gastric cancer metastasis. To destroy the basement membrane organization is the initial stage of invasion in tumor cells, which mainly depends on the IV collagen degradation enzymes, such as MMP2 and MMP9. The tumor activation of MMP2 and MMP9 is associated with metastasis and poor prognosis. Therefore, we further determined the expression of MMP2 and MMP9 in HIF2α knockdown SGC7901 cells and HIF2α overexpressing BGC823 cells. MMP2 and MMP9 both increased in BGC823 cells and decreased in SGC7901 cells. These findings suggest that HIF2α promotes the invasion and metastasis through upregulating MMP2 and MMP9 expression in gastric cancer cells.

Peritoneal dissemination not only occurs frequently but also mainly facilitates death in patients with advanced gastric cancer. Although peritoneal dissemination predicts poor prognosis of cancer, the molecular mechanism requires further study. *In vivo* experiments on animals, showed that HIF2α overexpressing BGC823 cells significantly improved the pulmonary metastasis of gastric cancer cells compared with no-load. This result accorded with the previous conclusion that HIF2α is significantly correlated with peritoneum metastasis in gastric cancer samples. Therefore, HIF2α plays an important role in the occurrence of peritoneal dissemination in gastric cancer.

In conclusion, we provide evidence that HIF2α is expressed in gastric cancer tissues, and significantly correlate with cancer clinical stage. These data strongly indicate that HIF2α affects the proliferation, invasion, metastasis promoting effects on gastric cancer cells, through a mechanism that, at least in part, involves the activation of Survivin, Cyclin D1, MMP2 and MMP9.

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