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Research article

Mechanism of HIF-1α promoting proliferation, invasion and metastasis of nasopharyngeal carcinoma by regulating MMP-2 in hypoxic microenvironment

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ABSTRACT

Objective: To explore the mechanism of HIF-1α promoting the proliferation, invasion and metastasis of nasopharyngeal carcinoma cells by regulating the expression of MMP-2.
Methods: 30 nasopharyngeal carcinoma tissues and 30 normal nasopharyngeal epithelial tissues were collected, and the expression of HIF-1α and MMP-2 in the nasopharyngeal carcinoma, normal nasopharyngeal epithelial tissues and their hypoxic environment were systematically analyzed by qRT-PCR and western blot techniques. Lentivirus transfection technology was used to regulate the expression of HIF-1α and MMP-2 genes in the HONE1 cell line under hypoxic environment, and to explore the interaction mechanism of HIF-1α and MMP-2 genes and their role in the proliferation, invasion and metastasis of nasopharyngeal carcinoma. Furthermore, the cytological behavior changes regulated by HIF-1α and MMP-2 genes were further explored by gene chip technology.
Results: The expressions of HIF-1α and MMP-2 in nasopharyngeal carcinoma tissues were significantly higher than those in normal nasopharyngeal epithelial tissues ($P < 0.05$). Compared with normoxic group, the expression of HIF-1α and MMP-2 in the nasopharyngeal carcinoma cell line HONE1 increased in hypoxic group ($P < 0.05$). Compared with NC-siRNA group, the expression of HIF-1α in si-HIF-1α group decreased, and the cell proliferation ability and invasion and metastasis ability decreased ($P < 0.05$). PCR array analysis revealed that the mRNA expressions of FAS, BRCA1, TIMP-1 genes were up-regulated in nasopharyngeal carcinoma HONE1 cells with HIF-1α gene silencing. AKT1, VEGFA, MET, MMP-2, MMP-9 and MTA2 were down-regulated. Compared with NC-siRNA group, the expression of MMP-2 in si-MMP-2 group decreased, and the ability of cell proliferation and invasion and metastasis decreased ($P < 0.05$).
Conclusion: HIF-1α could inhibit the proliferation, invasion and metastasis of nasopharyngeal carcinoma by regulating the expression of MMP-2, thus inhibiting tumor growth. Therefore, HIF-1α and MMP-2 might become important therapeutic targets to inhibit the growth, invasion and metastasis of nasopharyngeal carcinoma.

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Nasopharyngeal carcinoma (NPC) is a kind of squamous cell carcinoma originating from nasopharyngeal epithelial cells, which has obvious geographical distribution. The incidence rate is the highest in Guangdong and Guangxi [1–3]. At present, distant metastasis is the most common cause of treatment failure in patients with nasopharyngeal carcinoma [4]. Etiology of invasion and metastasis of nasopharyngeal carcinoma is still unclear, and its occurrence and development involves many factors [5,6]. The emerging tumor microenvironment (TME) is a complex and continuously evolving entity. The composition of the TME varies between tumor types. The nasopharyngeal carcinoma microenvironment includes nasopharyngeal carcinoma cells, tumor-associated fibroblasts, tumor-associated macrophages, immune cells, cytokines, and extracellular matrix, which are characterized by hypoxia, low pH, and high pressure [7]. Hypoxia is a characteristic pathophysiological feature of locally advanced solid tumors and a related factor in tumor physiology, which can promote tumor progression and treatment resistance. Hypoxia-inducible factor-1α (HIF-1α) is an important transcription factor of cells, which can regulate the response of cells to hypoxia. In normoxic environment, HIF-1α is at a low level, and if the body is in hypoxic environment, the expression of HIF-1α is significantly increased. HIF-1α is highly expressed in the hypoxic region of tumor tissue, which is related to the pathogenesis of many tumors. HIF-1α can promote the progress of nasopharyngeal carcinoma by regulating the expression of downstream target genes [8,9]. Through correlation analysis, it was found that HIF-1α and matrix metalloproteinase-2 (MMP-2) protein were positively correlated in breast cancer tissues, suggesting that they regulated each other and played a synergistic role in the occurrence, development, invasion and metastasis of breast cancer [10]. As a target gene downstream of HIF-1a, MMP-2 can degrade not only type IV collagen, but also type V collagen and laminin in the basement membrane, which greatly affects the permeability of basement membrane to tumor cells and therefore MMP-2 is closely related to tumor invasion and metastasis [11]. This study will explore the relationship between HIF-1α and the occurrence and development of nasopharyngeal carcinoma and its mechanism, in order to provide a basis for exploring new strategies for nasopharyngeal carcinoma treatment.

1. Materials and methods

1.1. Tissue samples, cell line and cell culture

30 nasopharyngeal carcinoma (NPC) tissues and 30 normal nasopharyngeal epithelial (NNE) tissue specimens were collected from the Department of Otolaryngology, Head and Neck Surgery, the Third Affiliated Hospital of Guangxi Medical University (Nanning, China). This study was approved by the Ethics Committee of the Third Affiliated Hospital of Guangxi Medical University (IR number: Y2024210, Approval date: 2024-04-07). All patients signed the informed consent form. Human nasopharyngeal carcinoma cell line HONE1 is preserved in our laboratory. HONE1 cells were maintained in DMEM high-sugar medium(Invitrogen, USA) containing 10 % fetal bovine serum(Gibco, USA), 100U/mL penicillin and 100U/mL streptomycin, and cultured in an ordinary incubator with 20%O_2, 5%CO_2 and 37 °C saturated humidity. After the cells were passaged and attached to the wall, they were placed in a hypoxic incubator with 1 % O_2, 5%CO_2, 94%N_2 and saturated humidity of 37 °C to simulate the hypoxic microenvironment in the tumor.

Table 1
Primer sequences used in this study.

Primer	Primer sequence (5′–3′)	Product size (bp)
HIF-1α	Forward: CCCAATGTCGGAGTTTGGAAAA Reverse: GACGTTCAGAACTTATCCTACCAT	117
MMP-2	Forward: GTGAAGTATGGGAACGCCGA Reverse: TGGTGAACAGGGCTTCATGG	178
FAS	Forward: CTGCCATAAGCCCTGTCCTCCA Reverse: ATTCTGGGTCCGGGTGCAGTT	199
BRCA1	Forward: ACCAACATGCCCACAGATCA Reverse: CACAGGTGCCTCACACATCT	180
AKT1	Forward: CTGCACAAACGAGGGGAGTA Reverse: TCACGTTGGTCCACATCCTG	113
VEGFA	Forward: ACATCACCATGCAGATTATGCG Reverse: GCTCCAGGGCATTAGACAGC	230
MET	Forward: TAGCCAACCGAGAGACAAGC Reverse: GCGATGTTGACATGCCACTG	197
MMP-9	Forward: TTCTGCCCGGACCAAGGATA Reverse: GACCATAGAGGTGCCGGATG	175
TIMP-1	Forward: CTGGCTTCTGGCATCCTGTT Reverse: CTTGGCCCTGATGACGAGGT	117
MTA2	Forward: TGAGAGTTGCCACACCACAC Reverse: GCGCTGGTTGTGTAAGGAGA	222
GAPDH	Forward: CCAGGTGGTCTCCTCTGACT Reverse: GCCAAATTCGTTGTCATACCAGG	121

HIF-1α Hypoxia-inducible factor-1α, *MMP-2* Matrix metalloproteinase 2, *FAS* Fas cell surface death receptor, *BRCA1* Breast cancer type1 susceptibility protein, *AKT1* AKT serine/threonine kinase 1, *VEGFA* Vascular endothelial growth factor A, *MET* Mesenchymal-epithelial transition, *MMP-9* Matrix metalloproteinase 9, *TIMP-1* Matrix metalloproteinase inhibitor, *MTA2* Metastasis-associated protein, *GAPDH* glyceraldehyde-3-phosphate dehydrogenase.

1.2. siRNA interference technology

HONE1 cells were inoculated into a 6-well cell culture plate and cultured in a 5%CO_2 incubator at 37 °C. When the cells grew to 70 %–80 %, HONE1 cell line of nasopharyngeal carcinoma with HIF-1α and MMP-2 silencing and their corresponding control groups were constructed by lentivirus method: si-HIF-1α group and NC-siRNA group, si-MMP-2 group and NC-siRNA group. After the cells were cultured for 48 h, the subsequent assay operations were carried out.

1.3. qRT-PCR assay

The tissues and cells were collected, the total RNA was extracted by Trizol (Invitrogen, USA) method, the RNA concentration of each sample was determined by micro-spectrophotometer (Thermo Fisher Scientific, USA), and the RNA was reverse transcribed into cDNA. According to the operating instructions, CFX96 TM Real-Time PCR detection system (Bio-Rad, USA) was used for Real-time PCR. Using GAPDH as internal reference, the relative expression level of gene was calculated by $2^{-\Delta\Delta Ct}$ method. The primer sequences (Invitrogen, USA) are shown in Table 1.

1.4. Western blot assay

Collect tissues and cells, add RIPA lysate (Beyotime, China), completely lyse on ice for 30min, collect supernatant by centrifugation, and determine the protein concentration of supernatant by BCA method (Beyotime, China). 50 μg of protein in each group was separated by SDS-PAGE (Bio-Rad, USA) and transferred to PVDF membrane (Millipore Sigma, USA) by wet transfer method. 10 % skimmed milk powder (solarbio, China) was sealed at room temperature for 2 h. The primary antibodies HIF-1α (1:1000, Abcam, USA), MMP-2 (1:1000, Cohesion Biosciences, UK), Fas (1:1000, Abcam, USA), BRCA1 (1:1000, Cohesion Biosciences, UK), TIMP-1 (1:1000, Abcam, USA), AKT1 (1:1000, Cohesion Biosciences, UK), VEGFA (1:1000, Abcam, USA), MET (1:1000, Cohesion Biosciences, UK), MMP-9 (1: 1000, Cohesion Biosciences, UK), MTA2 (1:1000, Cohesion Biosciences, UK), and β-actin (1:5000, Cohesion Biosciences, UK) were incubated overnight at 4 °C. The secondary antibody (anti-rabbit or anti-mouse, 1:5000, Cohesion Biosciences, UK) containing horseradish peroxidase (HRP) was incubated at room temperature for 1 h, and ECL luminescent liquid ((Beyotime, China) was dripped on the membrane, and the protein bands were detected by chemiluminescence imager (Bio-Rad, USA).

1.5. CCK-8 assay

Cell suspensions (3×10^4 cells/mL) were prepared from transfected nasopharyngeal carcinoma cells HONE1, which were inoculated into 96-well plates with 100μL/well, and each group was provided with 4 multiple wells. After the cells completely adhered to the wall, it was recorded as 0 h, and 10 μL CCK-8 solution (Beyotime, China) was added to each well at 0, 24, 48, 72 and 96 h respectively, and then gently mixed. The optical density (OD) at 450 nm was measured after 2 h incubation in the incubator in the dark, and the results were recorded and the cell growth curve was drawn.

1.6. Transwell assay

200 μL serum-free cell suspension containing 30,000 cells was inoculated into the upper chamber of Transwell chamber (BD, USA), and 600 μL complete culture medium containing 10 % fetal bovine serum was added into the lower chamber. After incubation in an incubator at 37 °C for 48 h, swab the chamber lightly to remove all cells that did not pass through the chamber. The cells migrated to the lower chamber were fixed with 90 % ethanol for 10 min, and then stained with 0.1 % crystal violet for 15 min. Wash off the excess dye and put it under a microscope (Olympus, Japan) to observe and take pictures.

1.7. Cancer PathwayFinder PCR array

PCR Array, also known as "functional classification chip", combines the advantages of sensitivity and reliability of real-time PCR technology and the advantages of microarray technology to detect the expression of multiple genes at the same time, and is used to analyze the signal pathway or the expression status of genes related to a biological function. Cancer PathwayFinder PCR Array can simultaneously determine the effects of transcription and expression of 84 key genes in six main signal pathways related to malignant tumors, and analyze the assay results with online data analysis program. Assay steps: the total RNA of cells was extracted, the RNA was reverse transcribed into cDNA, a PCR reaction system was prepared, a Real-time PCR assay was carried out, and online data analysis was carried out according to the gene arrangement on PCR-array (Qiagen, USA).

1.8. Statistical analysis

Statistical software SPSS26.0 (SPSS Inc., USA) was used to analyze the data, and the measurement data were expressed by mean ± standard deviation. T test showed that the difference was statistically significant with $P < 0.05$.

2. Results

2.1. HIF-1α is highly expressed in nasopharyngeal carcinoma tissues and nasopharyngeal carcinoma cell line HONE1

qRT-PCR and western blot were used to detect the expression of HIF-1α in 30 nasopharyngeal carcinoma tissues and 30 normal nasopharyngeal epithelial tissues. The results showed that the mRNA and protein expression level of HIF-1α in nasopharyngeal carcinoma tissues increased ($P < 0.05$) (Fig. 1A and B; Fig. S1 A). Compared with normoxic group, the expression level of HIF-1α mRNA and protein in nasopharyngeal carcinoma cell line HONE1 in hypoxic group increased ($P < 0.05$) (Fig. 1C and D; Fig. S1 B).

2.2. In hypoxic environment, silencing HIF-1α reduces the ability of cell proliferation, invasion and metastasis

qRT-PCR and western blot results showed that the expression of HIF-1α mRNA and protein in si-HIF-1α group was lower than that in si-NC group ($P < 0.05$) (Fig. 2A and B; Fig. S2 A).

The results of CCK-8 showed that, compared with si-NC group, the proliferation ability of HONE1 cells in si-HIF-1α group decreased significantly at 48, 72 and 96 h ($P < 0.05$) (Fig. 2C). Transwell invasion assay showed that compared with si-NC group, the number of HONE1 cells in si-HIF-1α group decreased at both 24 h and 48 h, and the decrease was more significant at 48 h ($P < 0.05$; Fig. 2D–G).

2.3. Effects of HIF-1α on the expression of mRNA related to apoptosis, cell cycle, angiogenesis, invasion and metastasis in nasopharyngeal carcinoma cells

After silencing HIF-1α in hypoxic environment, the transcription and expression changes of 84 key genes in 6 major tumor-related pathways in HONE1 cells were analyzed by Human Cancer PathwayFinder PCR Array. The results showed that the expression level of 9 genes changed more than 2-fold, of which 3 genes were up-regulated and 6 genes were down-regulated. Signal pathway of apoptosis and cell senescence: FAS (up-regulated by 2.16-fold); Signal pathway of cell cycle control and DNA damage repair: BRCA1 (up-regulated by 3.65-fold); Signal transduction and transcription signal pathway: AKT1 (down-regulated by 2.35-fold); Angiogenesis signal pathway: VEGFA (down-regulated by 2.86-fold); Invasion and metastasis signal pathway: MET (down-regulated by 2.06-fold), MMP-2 (down-regulated by 5.86-fold), MMP-9 (down-regulated by 2.21-fold), TIMP-1 (up-regulated by 2.18-fold) and MTA2 (down-regulated by 2.03-fold) (Table 2). In order to further determine the reliability of PCR Array results, we used qRT-PCR to verify the results, and the results showed that the trend was consistent with that of PCR Array analysis results (Fig. 3A). At the same time, we verified the protein level of Fas, BRCA1, TIMP-1, AKT1, VEGFA, MET, MMP-2, MMP-9 and MTA2 genes in PCR Array results by western blot, and the results showed that the trend was consistent with that in PCR Array analysis (Fig. 3B; Fig. S3 A-J). We selected MMP-2 with the most significant difference for further study.

2.4. MMP-2 is highly expressed in nasopharyngeal carcinoma tissues and nasopharyngeal carcinoma cell line HONE1

qRT-PCR was used to detect the expression of MMP-2 in 30 nasopharyngeal carcinoma tissues and 30 normal nasopharyngeal epithelial tissues. The results showed that the mRNA and protein expression level of MMP-2 in nasopharyngeal carcinoma tissues increased ($P < 0.05$) (Fig. 4A and B; Fig. S4 A, B). Compared with normoxic group, the expression level of MMP-2 mRNA and protein in nasopharyngeal carcinoma cell line HONE1 in hypoxic group increased ($P < 0.05$) (Fig. 4C and D; Fig. S4 C, D).

2.5. In hypoxic environment, silencing MMP-2 reduces the ability of cell proliferation, invasion and metastasis

qRT-PCR and western blot results showed that the expression of MMP-2 mRNA and protein in si-MMP-2 group was lower than that in si-NC group ($P < 0.05$) (Fig. 5A and B; Fig. S5 A, B).

The number of HONE1 cells decreased both at 72 h and 96 h in si-MMP-2 group, and the decrease was significant at 72 h ($P < 0.05$;

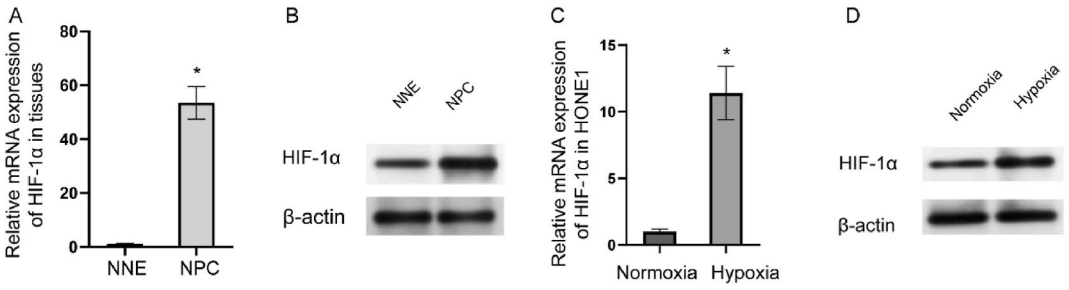

Fig. 1. The HIF-1α expression is increased in NPC. A. HIF-1α mRNA expression was tested by qRT-PCR in NPC tissues (n = 30) and NNE (n = 30). B. HIF-1α protein expression was tested by western blot in NPC tissues (n = 30) and NNE (n = 30). C. HIF-1α mRNA expression was tested by qRT-PCR under hypoxic/normoxic conditions in HONE1 D. HIF-1α protein expression was tested by western blot under hypoxic/normoxic conditions in HONE1. *$P < 0.05$; Full, non-adjusted images of the WB in (B) and (D) are shown in Fig. S1.

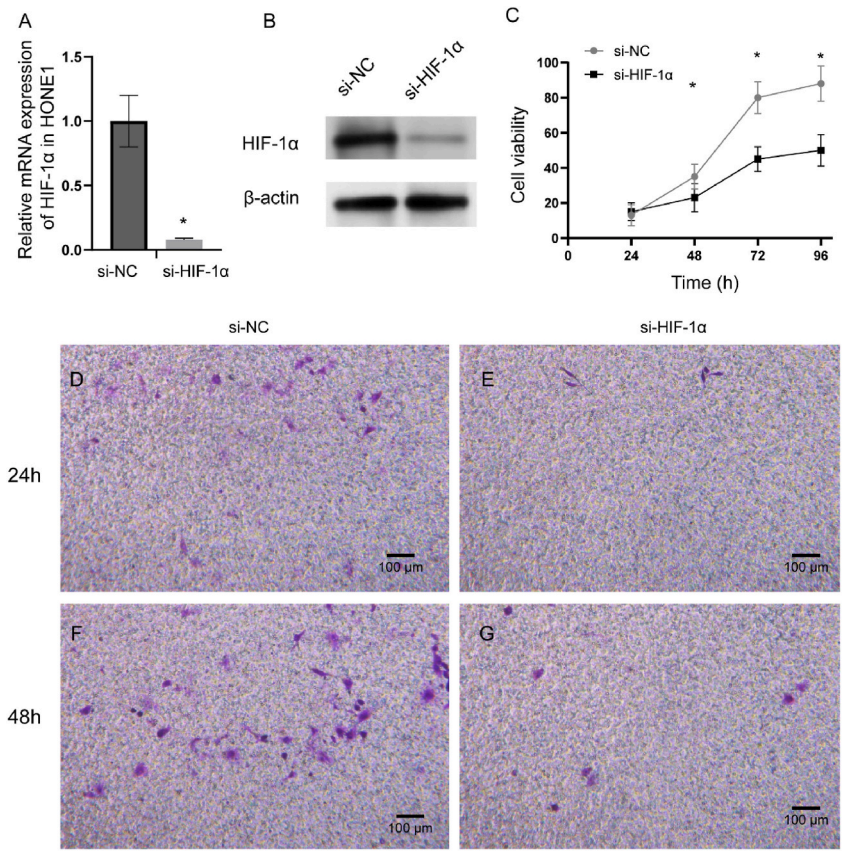

Fig. 2. Silencing HIF-1α reduces cell proliferation, invasion and metastasis under hypoxia. A. Effects of silencing HIF-1α on the expression of HIF-1α mRNA in HONE1. B. Effects of silencing HIF-1α on the expression of HIF-1α protein in HONE1. C. CCK-8 assay detecting the effect of silencing HIF-1α on HONE1 cell proliferation ability. D, E. Transwell detection of silencing HIF-1α on HONE1 cell migratory ability at 24 h. F, G. Transwell detection of silencing HIF-1α on HONE1 cell migratory ability at 48 h $^*P < 0.05$; Full, non-adjusted images of the WB are shown in Fig. S2.

Table 2
Genes up-regulated or down-regulated at least 2.0-fold by silencing HIF-1α in HONE1 cells.

Pathway	Gene symbol	Name of gene	Fold changed
Apoptosis and cell senescence	*FAS*	Fas cell surface death receptor	2.16
Cell cycle control & DNA damage repair	*BRCA1*	Breast cancer type1 susceptibility protein	3.65
Signal transduction and transcription	*AKT1*	AKT serine/threonine kinase 1	−2.35
Angiogenesis	*VEGFA*	Vascular endothelial growth factor A	−2.86
Invasion and metastasis	*MET*	Mesenchymal-epithelial transition	−2.06
	MMP-2	Matrix metalloproteinase 2	−5.86
	MMP-9	Matrix metalloproteinase 9	−2.21
	TIMP-1	Matrix metalloproteinase inhibitor	2.18
	MTA2	Metastasis-associated protein	−2.03

Fig. 5C). Transwell invasion assay showed that compared with si-NC group, the number of HONE1 cells in si-MMP-2 group decreased at both 24 h and 48 h, and the decrease was more significant at 48 h ($P < 0.05$; Fig. 5D–G).

3. Discussion

The local recurrence and distant metastasis of nasopharyngeal carcinoma are major obstacles in the clinical management of nasopharyngeal carcinoma [12]. The 5-year survival rate of patients is below 30 %, and the factors that lead to poor prognosis of nasopharyngeal carcinoma patients are complicated, which are closely related to the abnormal expression of gene proteins and the pathological characteristics of nasopharyngeal carcinoma [13]. Understanding the mechanisms of nasopharyngeal carcinoma, identifying biological markers for early diagnosis and new therapeutic targets has become an urgent scientific challenge in this field. Hypoxia can induce the expression of HIF-1α gene in tumor microenvironment. HIF-1 is a transcription regulatory factor existing in

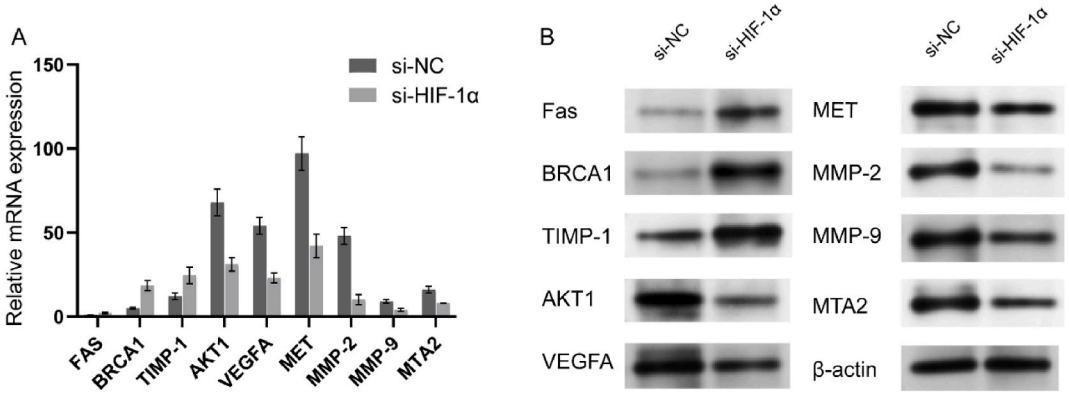

Fig. 3. Confirmation of genes regulated by HIF-1α in HONE1. A. qRT-PCR confirmation of genes regulated by HIF-1α in HONE1, including FAS, BRCA1, TIMP-1, AKT1, VEGFA, MET, MMP-2, MMP-9, MTA2. B. Western blot confirmation of genes regulated by HIF-1α in HONE1, including Fas, BRCA1, TIMP-1, AKT1, VEGFA, MET, MMP-2, MMP-9, MTA2. Full, non-adjusted images of the WB are shown in Fig. S3.

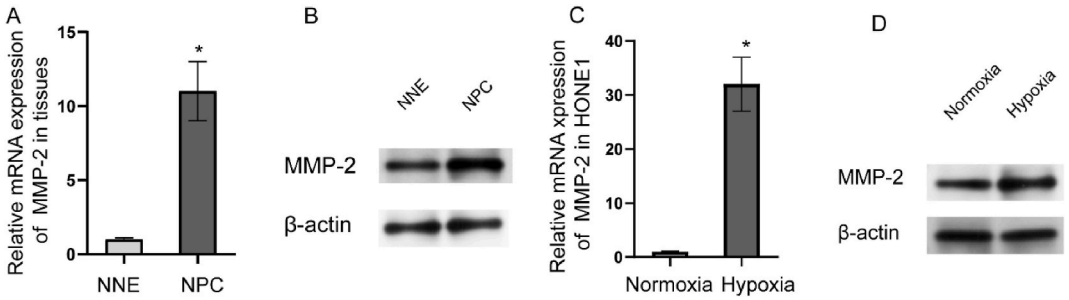

Fig. 4. The MMP-2 expression is increased in NPC. A. MMP-2 mRNA expression was tested by qRT-PCR in NPC tissues (n = 30) and NNE (n = 30). B. MMP-2 protein expression was tested by western blot in NPC tissues (n = 30) and NNE (n = 30). C. MMP-2 mRNA expression was tested by qRT-PCR under hypoxic/normoxic conditions in HONE1. D. MMP-2 protein expression was tested by western blot under hypoxic/normoxic conditions in HONE1. $^*P < 0.05$; Full, non-adjusted images of the WB are shown in Fig. S4.

human and mammals, located in the short arm of chromosome 14 (14q21-24). Its protein is composed of 120kD α subunit and 91-94kD β subunit, and exists as a heterodimer [14,15]. It is extremely unstable under normal oxygen partial pressure and is easily degraded by ubiquitin-proteasome pathway [14]. In anoxic environment, HIF-1α can stably accumulate and combine with HIF-1β to form heterodimers, which act on the anoxic response elements of the target gene to regulate the transcription process of downstream genes [16,17]. The activation of HIF-1α promotes the rapid adaptation of tumor cells to hypoxic environment, thus promoting the metastasis of various malignant tumors [18]. HIF-1a plays a key role in hypoxia, by stimulating the expression of key genes of tumor stem cells, thus maintaining their stemness. Studies have shown that silencing the expression of HIF-1α can effectively reduce the invasion ability of tumor cells, thus inhibiting the malignant progress of tumors [19].

In this study, the expression and regulation of HIF-1α and MMP-2 in tumor tissues and cells were deeply discussed by using molecular biology techniques such as Western blot and qRT-PCR. The results showed that the expression of HIF-1α and MMP-2 in the nasopharyngeal carcinoma tissues and metastatic lesions was significantly higher than that in normal tissues, and the high expression of HIF-1a was more common in metastatic lesions of nasopharyngeal carcinoma. Hypoxic environment could up-regulate the expression of HIF-1α in nasopharyngeal carcinoma cells, and the expression of MMP-2 gene increased with HIF-1α. HIF-1α is a key transcription factor for tumor progression and targeted therapy. The effect of HIF-1α depends on the presence or absence of oxygen. HIF-1α was completely inactivated and destroyed by ubiquitin proteasome pathway (UPP) in oxygen immersion environment. On the contrary, in anaerobic environment, it escaped destruction, entered the nucleus, and then up-regulated many genes related to cancer progress [15]. Over-expressed HIF-1α and its downstream genes support the progress of cancer through various mechanisms, including angiogenesis, cell proliferation and survival, metabolic reprogramming, invasion and metastasis, cancer stem cell maintenance, induced genetic instability and treatment resistance [15].

In the progress of cancer, HIF-1α may be activated by signal pathways unrelated to hypoxia. After silencing HIF-1α in hypoxic environment, we analyzed the transcription and expression changes of 84 key genes in 6 major tumor-related pathways in HONE1 cells by human Cancer PathwayFinder PCR Array. The results showed that the expression level of 9 genes changed more than 2-fold, among which 3 genes were up-regulated and 6 genes were down-regulated, among which MMP-2 was down-regulated by 5.86-fold, so we chose MMP-2 with the most significant differential expression for further study.

MMP-2 is a very important enzyme in MMPs. The human MMP-2 gene encodes a zymogen with a molecular weight of about 72 kDa,

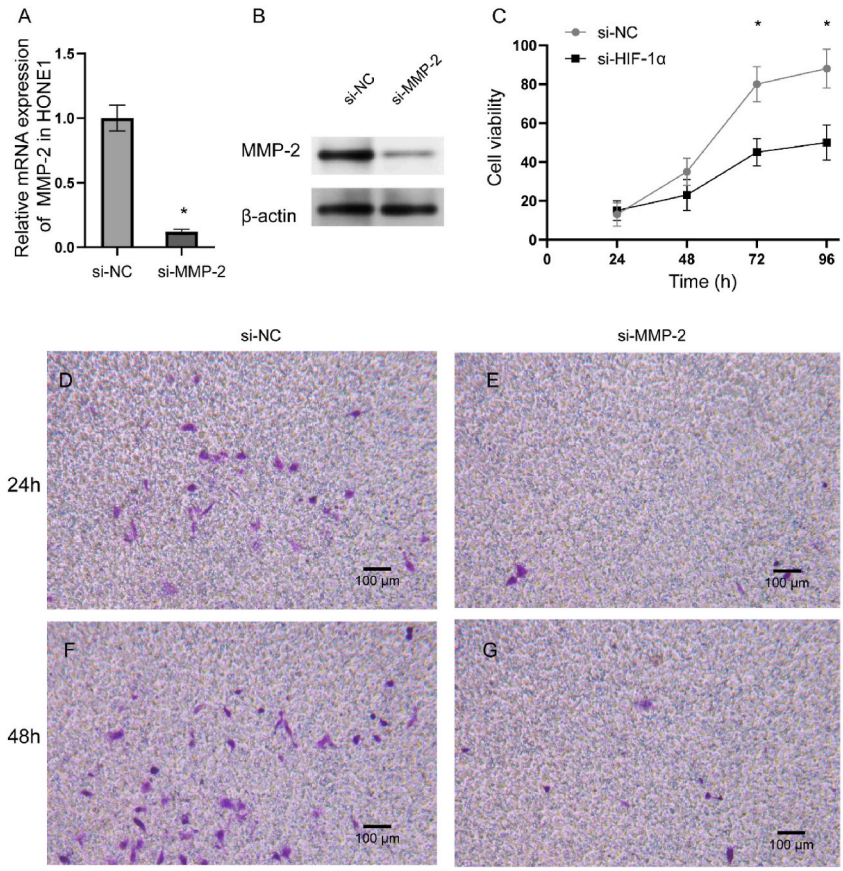

Fig. 5. Silencing MMP-2 reduces cell proliferation, invasion and metastasis under hypoxia. A. Effects of silencing MMP-2 on the expression of MMP-2 mRNA in HONE1. B. Effects of silencing MMP-2 on the expression of MMP-2 protein in HONE1. C. CCK-8 assay detecting the effect of silencing MMP-2 on HONE1 cell proliferation ability. D, E. Transwell detection of silencing MMP-2 on HONE1 cell migratory ability at 24 h. F, G. Transwell detection of silencing MMP-2 on HONE1 cell migratory ability at 48 h $^*P < 0.05$; Full, non-adjusted images of the WB are shown in Fig. S5.

which is hydrolyzed into an active form of 65 kDa after activation [11]. MMP-2 proenzyme consists of five parts: signal peptide domain, propeptide domain, catalytic domain, hinge domain and carboxyl terminal domain [11]. The expression level of MMP-2 in normal adult tissues is relatively low, and it will only increase when the system is stimulated or under pathological conditions. In physiological state, MMP-2 is involved in ovulation, neovascularization, tissue reconstruction and injury repair. Under pathological conditions, MMP-2 is involved in the progression of malignant tumor metastasis, acute coronary syndrome and other diseases [20]. Our study found that the mRNA expression level and protein expression level of MMP-2 in nasopharyngeal carcinoma tissues increased ($P < 0.05$). Compared with normoxic group, the expression level of MMP-2 mRNA and protein in nasopharyngeal carcinoma cell line HONE1 in hypoxic group increased.

Under hypoxia, silencing HIF-1α can inhibit the proliferation, invasion and metastasis of nasopharyngeal carcinoma HONE1 cells by regulating MMP-2. This discovery strongly suggests that HIF-1α plays an important role in the transcription regulation of MMP-2. The invasion and metastasis potential of tumor cells is positively correlated with their ability to express MMP-2, and higher expression of MMP-2 often means that tumors have stronger invasion and metastasis ability [21]. Li et al. performed immunohistochemical analysis on 144 nasopharyngeal carcinoma specimens, and found that there was high expression of MMP-2 in nasopharyngeal carcinoma specimens, and its expression level was positively correlated with T and M stages of the tumor [22]. Further analysis of the mechanism of MMP-2 showed that MMP-2 could degrade extracellular matrix and destroy important physical barriers such as basement membrane, thus reshaping the adhesion between cells and promoting the formation of tumor neovascularization [11,23,24]. These processes provide favorable conditions for tumor invasion and metastasis.

In this study, it was found that silencing HIF-1α affected the transcription of several main pathways related to tumor, including apoptosis, cell cycle, signal transduction, angiogenesis, tumor invasion and metastasis. Secondly, silencing HIF-1α promoted the apoptosis of nasopharyngeal carcinoma cells by up-regulating the expression of FAS gene, and promoted the proliferation of nasopharyngeal carcinoma cells by up-regulating the expression of BRCA1 gene. Most importantly, silencing HIF-1α inhibited the invasion and metastasis of nasopharyngeal carcinoma cells by up-regulating TIMP-1 and down-regulating the expression of MMP-2, MET, MMP-9 and MTA2. It was found that HIF-1α could promote the invasion and metastasis of hypopharyngeal squamous cell carcinoma FaDu cells and the high expression of MMP-2 in hypoxic environment [25]. In esophageal cancer, rich oxygenation and significant inhibition

of HIF-1α expression could reduce the expression of MMP-2 and further improve the radiosensitivity [26,27]. In addition, the latest research showed that HIF-1α accumulated in the nucleus under the condition of macrophage pathological changes, which further promoted the high expression of MMP-2 and MMP-9 [28]. These findings provided a new perspective for further understanding the role of HIF-1α in the occurrence and development of diseases. In this study, we found that silencing HIF-1α could effectively inhibit the expression of MMP-2 mRNA and protein, especially in inhibiting the expression of MMP-2 mRNA. Through in-depth analysis of the reasons, it was found that the regulation mechanism of MMP-2 was quite complicated, and there might be significant differences before and after transcription. Earlier studies showed that the gene promoter or enhancer activated by HIF-1 usually contains a DNA sequence less than 100bp, which was called hypoxia response element (HRE), and it mediated the response of cells to hypoxia [29]. However, this sequence was not found in the promoter of MMP-2, so it was speculated that the regulation of MMP-2 by HIF-1α might be indirect. Of course, the possibility that there are other unknown mechanisms cannot be ruled out. These findings provide a new perspective for a deeper understanding of the interaction between HIF-1α and MMP-2, and also provide potential targets for future therapeutic strategies for related diseases.

Limitations of this study include the relatively small sample size, which may limit the generality of the findings. In addition, the study may rely on in vitro experiments, which may not fully translate to the complexity of the tumor microenvironment in vivo. Future studies should aim to include larger cohorts and clinical data to confirm these findings, as well as to establish animal xenograft models that can also be tested in vivo.

To sum up, HIF-1α inhibits tumor growth by regulating the expression of MMP-2 and suppressing the proliferation, invasion and metastasis of nasopharyngeal carcinoma. Therefore, HIF-1α and MMP-2 may become important therapeutic targets for inhibiting nasopharyngeal carcinoma growth and invasion and metastasis.

CRediT authorship contribution statement

Ying Lan: Writing – review & editing, Writing – original draft, Funding acquisition, Conceptualization. **Shijie Zhao:** Methodology. **Tao Hou:** Funding acquisition, Formal analysis, Data curation. **Yi Ren:** Methodology. **Jun Tang:** Resources. **Shihua Yin:** Writing – review & editing, Visualization, Validation, Supervision, Funding acquisition. **Yang Wu:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Funding acquisition, Data curation.

Data availability statement

All of the data used to support the findings of this study are available from the corresponding author upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e40760.

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