RESEARCH ARTICLE

Hypoxia Induced Multidrug Resistance of Laryngeal Cancer Cells via Hypoxia-inducible Factor-1α

Da-Wei Li[1], Pin Dong[2], Fei Wang[2], Xin-Wei Chen[2], Cheng-Zhi Xu[3], Liang Zhou[1]*

Abstract

Objectives: To investigate whether hypoxia has an effect on regulation of multidrug resistance (MDR) to chemotherapeutic drugs in laryngeal carcinoma cells and explore the role of hypoxia-inducible factor-1α (HIF-1α). Methods: Laryngeal cancer cells were cultured under normoxic and hypoxic conditions. The sensitivity of the cells to multiple drugs and levels of apoptosis induced by paclitaxel were determined by MTT assay and annexin-V/propidium iodide staining analysis, respectively. HIF-1α expression was blocked by RNA interference. The expression of HIF-1α gene was detected by real-time quantitative RT-PCR and Western blotting. The value of fluorescence intensity of intracellular adriamycin accumulation and retention in cells was evaluated by flow cytometry. Results: The sensitivity to multiple chemotherapy agents and induction of apoptosis by paclitaxel could be reduced by hypoxia ($P<0.05$). A the same time, the adriamycin releasing index of cells was increased ($P<0.05$). However, resistance acquisition subject to hypoxia *in vitro* was suppressed by down-regulating HIF-1α expression. Conclusion: HIF-1α could be considered as a key regulator for mediating hypoxia-induced MDR in laryngeal cancer cells via inhibition of drug-induced apoptosis and decrease in intracellular drug accumulation.

Keywords: Cell hypoxia - hypoxia inducible factor-1α - drug resistance, multiple - laryngeal neoplasms

Asian Pac J Cancer Prev, **14 (8)**, 4853-4858

Introduction

Laryngeal carcinoma is the second most common malignancy in the head and neck region (Chu et al., 2008). Except for invasion and metastasis, multidrug resistance (MDR) is a major reason related to the failure of treatment in human laryngeal cancer. Therefore, the mechanisms underlying MDR in laryngeal cancer cells are attracting extensive attention. So far, the pathogenetic mechanisms that regulate MDR in laryngeal carcinoma cells are still not well known.

It is well-known that the rapid proliferation of malignant cells and the irregular local vasculature jointly favor the formation of hypoxic areas within human solid tumors including laryngeal cancer. Hypoxia subjects cells to a series of functional adaptive responses through both gene regulation and post-transcriptional modification of certain proteins (Wang et al., 1995). One of multiple adaptive behaviors is that hypoxic cells clearly come to resist chemotherapy in various types of cancer, such as lung cancer (Wohlkoenig et al., 2011), oral cancer (Yoshiba et al., 2009) and ovarian cancer (Huang et al., 2010). Unfortunately, the detailed mechanisms for chemotherapy resistance of hypoxic cells are still not fully understood. To our knowledge, there are currently no studies that focus on the relationship between hypoxia and MDR in human laryngeal carcinoma cells.

The adaptive response of tumor cells to hypoxia conditions is dependent on a series of regulators. Hypoxia-inducible factor-1 (HIF-1), which is generally considered as a critical molecule for hypoxic cells to experience the hypoxic adaptive alterations by regulating the transcription of a number of target genes (Li et al., 2010). HIF-1 consists of HIF-1α and HIF-1β subunits. In contrast to the constitutively expressed HIF-1β, HIF-1α stability and synthesis is strictly regulated by cellular oxygen levels and growth factors stimulation (O'Donnell et al., 2006). HIF-1α has been regarded as a key element to mediate the function of HIF-1 to activate a set of hypoxia-inducible genes, regulating tumor angiogenesis (Park et al., 2010), metastasis (Jing et al., 2012), resistance to therapy (Zhu et al., 2005; Huang et al., 2010) and other adaptations to hypoxia. Recently, several literatures have reported that overexpression of HIF-1α protein is significantly correlated with local recurrence, tumor progression and metastasis in human laryngeal carcinoma (Schrijvers et al., 2008; Wu et al., 2010), suggesting that HIF-1α might serve as a determinant of malignant biological behaviors

*[1]Department of Otolaryngology-Head and Neck Surgery, Affiliated Eye and ENT Hospital of Fudan University, [2]Department of Otolaryngology-Head and Neck Surgery, Shanghai Jiaotong University Affiliated First People's Hospital, [3]Department of Otolaryngology-Head and Neck Surgery, Shanghai Ninth People's Hospital Affiliated Shanghai Jiaotong University School of Medicine, Shanghai, China *For correspondence: zhouliang_lidawei@163.com*

of laryngeal cancer cells. However, it is far from clear whether HIF-1α is responsible for the occurrence of MDR in laryngeal cancer cells.

In our study, we investigated the regulation of MDR by hypoxia in laryngeal carcinoma cells. Meanwhile, we also explored the role of HIF-1α in hypoxia-induced resistance to chemotherapy drugs of laryngeal cancer cells and its possible mechanisms

Materials and Methods

Cell line and culture

The laryngeal carcinoma cells Hep-2 was gained from the American Type Culture Collection (ATCC) and multidrug-resistant cell variant Hep-2T had been prepared and characterized in our previous work (Li et al., 2007). All cell lines were maintained as monolayers in Dulbecco's modification of Eagle's medium (DMEM; Gibco Corporation, USA), supplemented with 10% fetal bovine serum (Hyclone, USA) and antibiotics (100 IU/ml penicillin and 100 IU/ml streptomycin) at 37°C in a humidified atmosphere containing 95% air and 5% CO_2.

Exposure to hypoxia

For hypoxic exposure, tumor cells were cultured in a modulator incubator chamber (NuaireTM US autoflow CO_2 water jacketed incubator) at 37°C with 94% N2, 1% O2 and 5% CO_2.

Inhibition of HIF-1α expression by RNA interference

The double strand siRNA oligonucleotide targeting human HIF-1α gene (sense: 5-CUGAUGACCAGCAACUUGAdTdT-3, antisense: 5-UCAAGUUGCUGGUCAUCAGdTdT-3) was synthesized by Shanghai Genepharma Co. Ltd. (China), which was confirmed previously (Sowter et al., 2003). Meanwhile, a nonspecific control siRNA (sense: 5-AGUUCAACGACCAGUAGUCdTdT-3, antisense: 5-GACUACUGGUCGUUGAdTdT-3) was also synthesized by Genepharma Company, which was identified no homology to any human transcripts in records. Laryngeal carcinoma cells should be cultured in antibiotics-free medium for 24 hours before transfection with siRNA (100 nM) using Lipofectamine 2000. After transfection for 24 hours, the cells could be harvested and examined.

Real-time quantitative RT-PCR (QRT-PCR) for HIF-1α

Total RNA was extracted from Hep-2 and Hep-2T cells using Trizol reagent (Invitrogen) according to the manufacturer's instructions. Then, the cDNA was synthesized from the isolated RNA by the reverse transcription reaction (RT), which was performed as in our previous study (Li et al., 2013). HIF-1α primers were 5- AACATAAAGTCTGCAACATGGAAG-3 (sense) and 5- AACATAAAGTCTGCAACATGGAAG-3 (antisense). GAPDH (internal control) primers were 5-CATCTTCCAGGAGCGAGA-3 (sense) and 5-TGTTGTCATACTTCTCAT-3 (antisense). PCR amplification was performed in a 20 μL final reaction mixture including a diluted complementary DNA (cDNA) solution, 10 μM of each primer, and 10 μL SYBR Green PCR Master Mix. The thermal cycling conditions were shown as the following: one cycle at 95˚C for 10 minutes, 40 cycles at 95˚C for 15 seconds and at 60˚C for 60 seconds. The data of QRT-PCR were analyzed by the $2^{-\triangle\triangle CT}$ method (Livak et al., 2001).

Western blot analysis for HIF-1α

After appropriate treatment according to experimental demand, laryngeal cancer cells were harvested and lysed with a cold radioimmune precipitation buffer (RIPA) protein lysis buffer for 30 minutes. Then, equal amounts of protein extracts (25 μg) were loaded onto sodium dodecyl sulfate polyacrylamide gel electropheresis (SDS-PAGE, 5% stacking gel and 8% separating gel for HIF-1α), followed with a separation at 80 V for about 2 hours and transferred onto a polyvinylidene difluoride (PVDF) membrane (Millipore), blocked overnight with 4% skimmed milk for 1.5 hours at room temperature. After that, they were incubated with primary antibodies overnight at 4˚C (HIF-1α 1:100, mouse anti-human; β-actin, 1:1000, rabbit anti-human) followed by horseradish peroxidase-conjugated anti-rabbit or anti-mouse secondary antibodies (1: 2000; room temperature, 1 hour). Finally, labeled bands from washed blots were detected by electrogenerated chemiluminescence (ECL) according to the manufacturer's instructions.

Analysis of multidrug resistance of laryngeal cancer cells

The sensitivity of laryngeal cancer cells to 5-FU, cisplatin, adriamycin, paclitaxel and gemcitabine was measured by 3-(4, 5-dimthylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) assay, and cells were plated in 96-well culture panels (5×10^3 cells/well). 12 hours later, cells were treated with relevant doses of chemotherapy agents and cultivated for 48 hours under normoxic or hypoxic conditions. The sensitivity of cells to different agents was tested with MTT assay, and the concentration of each drug that caused a 50% reduction in the number of cells (IC_{50}) was calculated according to the manufacturer's protocal. Each experiment was operated at least three times.

Annexin-V/propidium iodide staining

The apoptotic indices (AI) of laryngeal cancer cells were detected by flow cytometry (FCM). In brief, cells in the log phase were plated into six-well plates (4×10^5 cells/well) and cultured overnight at 37˚C. Then, culture medium was renewed and incubated in normoxia or hypoxia for 12 hours. Paclitaxel was added to each well until a final concentration of 2.5×10^{-9} M was reached. Cell culture was continued for 48 hours. After that, 5 μL (50 μg/ml) of Annexin-V-FITC were added to these cells, and cells were incubated for further 10 minutes. After washed twice in DMEM, cells were resuspended in 190 μL of Tris-HCl buffer. Then 10 μL (20 μg/ml) of propidium iodide (PI) were added to the resuspended cells and cultured at 4˚C for further 10 minutes. The mean fluorescence intensity of Annexin-V-FITC/PI was detected by FCM. Finally, the apoptosis rate was calculated at the mean fluorescence intensity.

Table 1. Comparison of Chemosensitivity in Hypoxic and Nomorxic Hep-2 Cells

Drug	IC_{50} (μg/ml)		Fold-resistance
	Normoxic cells	Hypoxic cells	
Paclitaxel	$4.46\times10^{-3}\pm0.37$	$37.89\times10^{-3}\pm0.3$*	8.53±0.75
5-Fu	32.19±0.5	242.64±1.66*	7.54±0.12
Doxorubicin	1.25±0.09	3.91±0.21*	3.13±0.06
Gemcitabine	14.45±0.47	38.60±0.64*	2.67±0.06
Cisplatin	2.52±0.25	8.79±0.51*	3.5±0.14

IC_{50} is the concentration of each drug that caused a 50% reduction in the number of cells; Mean ± SD of three individual experiments are shown; *P<0.05 vs. Nomorxic

Table 2. Comparison of Chemosensitivity in Hypoxic and Nomorxic Hep-2T Cells

Drug	IC_{50} (μg/ml)		Fold-resistance
	Normoxic cells	Hypoxic cells	
Paclitaxel	0.37±0.32	4.14±1.96*	11.13±0.69
5-Fu	81.46±0.61	687.69±4.39*	8.44±0.45
Doxorubicin	55.32±0.49	271.44±7.54*	4.91±0.20
Gemcitabine	28.55±0.56	107.45±6.24*	3.76±0.18
Cisplatin	3.72±0.16	18.70±0.16*	5 .04±0 .18

IC_{50} is the concentration of each drug that caused a 50% reduction in the number of cells; Mean ± SD of three individual experiments are shown; *P<0.05 vs. Nomorxic

Fluorescence intensity assay of intracellular adriamycin
The fluorescence intensity of intracellular adriamycin (ADM) was assessed by FCM. Briefly, cells in the log phase were seeded into six-well plates (4×10^5 cells/well). After cultured overnight at 37˚C, the culture medium was replaced with serum deprived DMEM medium and incubated with continuous normoxia or hypoxia for 12 hours. Then, cells were cultured for 1 hour after the addition of ADM to reach a final concentration of 5 mg/L. Next, the cells were harvested to test ADM accumulation and, alternatively, cultured in drug-free DMEM for another 30 minutes, followed to detect ADM retention. Finally, the fluorescence intensity of intracellular ADM was detected by FCM after cells had been washed twice with ice-cold phosphate-buffered saline (PBS). The ADM-releasing index of laryngeal cancer cells was calculated according to the following formula: releasing index = (accumulation value-retention value)/accumulation value.

Statistical analysis

Our data were expressed as mean ± standard deviation (SD). Comparisons of quantitative variables were valuated by Student's t-test or one-way ANOVA analysis with SPSS13.0 statistical software (SPSS Inc, Chicago, Illinois, USA). Probability value of less than 0.05 was considered statistically significant.

Results

Hypoxia increased multidrug resistance of laryngeal carcinoma cells

The sensitivity of laryngeal cancer cells to multiple categories of chemotherapeutic drugs was assessed by MTT assay under normoxic and hypoxic conditions. The

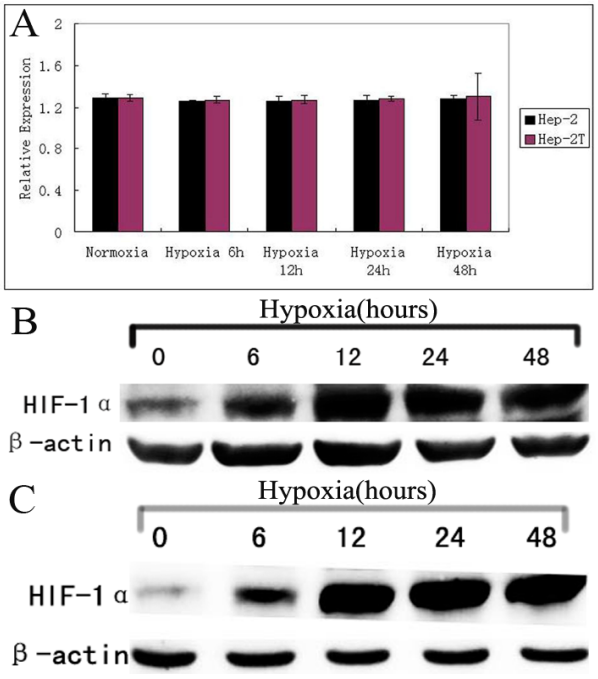

Figure 1. Expression of HIF-1α Gene Under Normoxic and Hypoxic Conditions. The relative expression of HIF-1α mRNA (A) to GAPDH mRNA were detected by Real-time quantitative RT-PCR. The expression of HIF-1α protein in Hep-2 (B) and Hep-2T (C) cells was determined by Western blot analysis

chemotherapy drugs tested were all less effective under hypoxic conditions than that of normoxia in both drug-sensitive cells Hep-2 (P<0.05; Table 1) and drug-resistant cells Hep-2T (P<0.05; Table 2). Both Hep-2 and Hep-2T cells exhibited a stronger drug resistance in hypoxia.

Hypoxia up-regulated expression of HIF-1α protein in laryngeal carcinoma cells

When laryngeal cancer cells were cultured under hypoxic conditions for 6, 12, 24, or 48 hours, the expression of HIF-1α were determined by QRT-PCR and Western blot. As shown in Figure 1A, there were no obvious difference in the expression of HIF-1α mRNA between hypoxic group and normoxic group of Hep-2 and Hep-2T cells (P>0.05). Western blot analysis revealed that the expression of HIF-1α protein in Hep-2 and Hep-2T cells had a time-dependent increase when cells were exposed to hypoxia, and reached a peak value at 24 hours under hypoxic conditions (P<0.05; Figure 1B and 1C).

Inhibition of HIF-1α expression suppressed multidrug resistance of hypoxic laryngeal cancer cells

In order to explore the role of HIF-1α in hypoxia-induced multidrug resistance, both Hep-2 and Hep-2T cells had been transfected with either a double strand siRNA oligonucleotide targeting HIF-1α gene (HIF-1α-siRNA) or nonspecific control siRNA for 24 hours before incubation under hypoxic conditions. As can be seen in Figure 2, both mRNA and protein expression of HIF-1α in hypoxic laryngeal cancer cells were obviously down-regulated after transfected with HIF-1α-siRNA in comparison to the negative control or untreated control (P<0.05). Then, to evaluate whether siRNA-directed

Table 3. Effect of HIF-1α Silencing on Chemosensitivity in Hypoxic Hep-2 Cells

Drug	IC_{50} (μg/ml)		
	Untreated control	Negative control	HIF-1α-siRNA
Paclitaxel	$38.83\times10^{-3}\pm0.48$	$38.40\times10^{-3}\pm0.87$	$9.35\times10^{-3}\pm0.68$*
5-Fu	242.57±0.97	240.89±0.92	49.80±1.98*
Doxorubicin	3.82±0.08	3.81±0.15	2.08±0.13*
Gemcitabine	38.51±0.65	38.52±0.35	20.45±0.93*
Cisplatin	8.75±0.12	8.69±0.27	3.55±0.12*

IC_{50} is the concentration of each drug that caused a 50% reduction in the number of cells; Mean ± SD of three individual experiments are shown; *P<0.05 vs. Untreated control and Negative control

Table 4. Effect of HIF-1α Silencing on Chemosensitivity in Hypoxic Hep-2 Cells

Drug	IC_{50} (μg/ml)		
	Untreated control	Negative control	HIF-1α-siRNA
Paclitaxel	4.17±0.89	4.14±1.21	0.60±0.18*
5-Fu	673.25±10.37	674.02±2.18	124.2±3.31*
Doxorubicin	268.44±2.58	265.13±7.71	75.21±1.90*
Gemcitabine	117.32±3.59	117.01±1.07	37.73±0.69*
Cisplatin	18.92±0.29	18.97±0.13	4.48±0.13*

IC_{50} is the concentration of each drug that caused a 50% reduction in the number of cells; Mean ± SD of three individual experiments are shown; *P<0.05 vs. Untreated control and Negative control

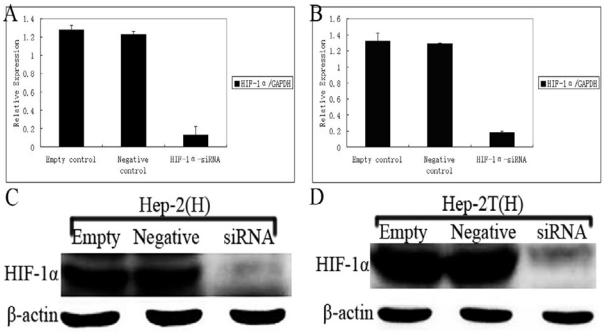

Figure 2. Downregulated HIF-1α Gene Expression in Hypoxic Laryngeal Cancer Cells Transfected with HIF-1α-siRNA. The expression of HIF-1α mRNA in Hep-2 (A) and Hep-2T (B) cells was analyzed by Real-time quantitative RT-PCR. HIF-1α protein expression in Hep-2 (C) and Hep-2T (D) cells were detected by Western blot. H means Hypoxia

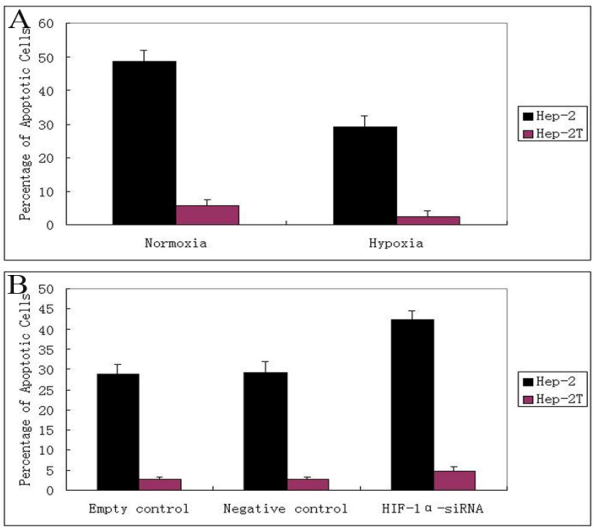

Figure 3. Effects of Hypoxia and HIF-1α on Apoptosis Induced by Paclitaxel. (A) The percentage of apoptotic Hep-2 and Hep-2T cells in normoxia or hypoxia pretreated with paclitaxel (2.5×10^{-9} M). *P<0.05: cells in hypoxia versus nomorxia. (B) The apoptosis rate in control groups and HIF-1α-siRNA group pretreated with paclitaxel (2.5×10^{-9} M). *P<0.05: cells/HIF-1α-siRNA versus cells/controls in hypoxia

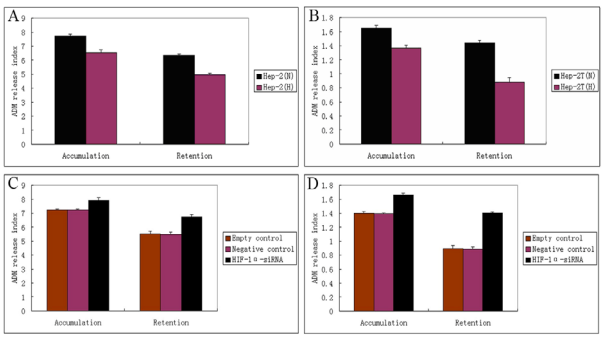

Figure 4. Effects of Hypoxia and HIF-1α on Fluorescence Intensity of Intracellular ADM. (A) Hep-2 cells in hypoxia or normoxia. *P<0.05: Hep-2/hypoxia versus Hep-2/nomorxia. (B) Hep-2T cells in hypoxia or normoxia. *P<0.05: Hep-2T/hypoxia versus Hep-2T/nomorxia. (C) Hep-2/ controls and Hep-2/ HIF-1α-siRNA cells in hypoxia. *P<0.05: Hep-2/HIF-1α-siRNA versus Hep-2/controls. (D) Hep-2T/ controls and Hep-2T/HIF-1α-siRNA cells in hypoxia. *P<0.05: Hep-2T/HIF-1α-siRNA versus Hep-2T/controls. N means Normoxia and H means Hypoxia

suppression of HIF-1α expression sensitized hypoxic cells to cytotoxic agents, we compared the drug sensitivity of the siRNA-treated to that of the mock-treated cells using MTT assay. The data showed that the sensitivity of hypoxic laryngeal cancer cells to paclitaxel, doxorubicin, gemcitabine, 5-FU, and cisplatin was significantly up-regulated by inhibiting the expression of HIF-1α protein (P<0.05; Table 3 and 4).

HIF-1α protects hypoxic laryngeal cancer cells from apoptosis induced by paclitaxel

As shown in Figure 3A, FCM with Annexin-V/PI staining indicated that the percentages of Hep-2 and Hep-2T cells apoptosis induced by paclitaxel were much less in hypoxia compared with normoxia (29.18%±3.12% vs. 48.72%±3.21%, P<0.05; 5.63%±1.71% vs. 2.52%±1.69%, P<0.05; respectively). Moreover, to assess the effect of HIF-1α in hypoxic protection of Hep-2 and Hep-2T cells from apoptosis induced by paclitaxel, Annexin V/PI staining assay revealed that cells transfected with HIF-1α-siRNA could obviously enhance drug-induced apoptosis in hypoxia (P<0.05; Figure 3B).

HIF-1α decreased intracellular ADM accumulation and retention

The effects of HIF-1α on intracellular drug accumulation and retention in hypoxic laryngeal cancer cells were evaluated using ADM. The fluorescence intensity of intracellular ADM was detected by FCM and the ADM-releasing index was then calculated. As shown in Figure 4A and 4B, laryngeal cancer cells subjected to hypoxia showed a significantly decreased ADM accumulation and retention, as well as an increased releasing index (P<0.05). Furthermore, downregulation of

HIF-1α expression in hypoxic laryngeal cancer cells by transfected with HIF-1α-siRNA could obviously enhance the accumulation and retention of ADM, as well as reduce the release of ADM (P<0.05; Figure 4C and 4D).

Discussion

Regional microenvironment hypoxia could be commonly observed in human solid tumors. Up to date, a mounting body of evidence has confirmed that the hypoxic microenvironment is associated with invasion (Huang et al., 2013) and metastasis (Zhang et al., 2013) of tumors. Additionally, a series of studies have demonstrated that the effects of chemotherapy could be reduced by hypoxia in certain tumors (Zhu et al., 2005; Liu et al., 2008; Huang et al., 2010), which has become a main obstacle in the development of effective anticancer therapy. To further determine whether hypoxia has an influence on multidrug resistance (MDR) of laryngeal cancer cells, we compared the drug sensitivity to 5-FU, cisplatin, adriamycin, paclitaxel and gemcitabine - five common chemotherapy drugs in treating human laryngeal cancer - of hypoxic Hep-2 and Hep-2T cells with that of nomorxic cells. Consequently, we found that all tested drugs were less effective in hypoxic laryngeal cancer cells than in nomorxic cells, almost in line with previous literatures about other cancer (Zhu et al., 2005; Liu et al., 2008). It has been elucidated that hypoxia could disturb the therapies against human malignancies by decreasing the effects of various chemotherapeutic drugs.

The hypoxic acquisition of MDR has been confirmed with respect to induction of some genes expression, which has been related to drug resistance. Nevertheless, the key transcriptional regulator that modulates the expression of drug-resistance-associated genes in hypoxic laryngeal cancer cells still remains to be identified. HIF-1α has been considered as a major transcriptional factor, which has been shown to be involved in the regulation of drug resistance acquired by hypoxia (Zhu et al., 2005; Liu et al., 2008; Huang et al., 2010). Consistent with the results of Wu et al. (2010), our previous study has confirmed that HIF-1α expression was highly expressed in human laryngeal carcinoma tissues and significantly correlated with tumor stage and lymph node metastasis, elucidating that HIF-1α may have effect on the progression of laryngeal cancer. Equally important, the present data demonstrated that the expression of HIF-1α protein in Hep-2 and Hep-2T cells was obviously enhanced by hypoxia, supporting the notion that HIF-1α is a hypoxia-dependent regulator of laryngeal cancer cells. Moreover, our study showed that blockage of HIF-1α expression could significantly up-regulate the sensitivity of laryngeal cancer cells to the above-mentioned drugs - 5-FU, cisplatin, adriamycin, paclitaxel and gemcitabine - under hypoxic environments. That is to say, HIF-1α mediates hypoxia-induced MDR in laryngeal cancer cells, and knockdown of HIF-1α expression might be regarded as an available method to reverse the MDR of human laryngeal cancer.

The active HIF-1α conferred MDR in hypoxic laryngeal cancer cells. However, the intrinsic mechanisms that arouse above - mentioned phenomena have not yet been devised. Our analytical data clearly showed that hypoxia could reduce the apoptosis of laryngeal cancer cells induced by paclitaxel. Further study from transfected cells has indicated that inhibition of HIF-1α expression lead to an increase in apoptosis of laryngeal cancer cells under hypoxic conditions. Almost consistent with the results of Liu et al. (2008) and Tak et al. (2011), our results elucidated that HIF-1α might partly be involved in hypoxia-induced MDR in laryngeal cancer cells via suppressing drug-induced apoptosis. The underlying mechanisms of HIF-1α in suppression of apoptosis in laryngeal cancer cells induced by chemotherapeutic drugs remains to be further determined. Besides, it has already been confirmed that the enhancement of drug transport function is another important mechanism of MDR acquired by hypoxia in several kinds of tumor cells (Thews et al., 2011; Chou et al., 2012). The present study demonstrated that hypoxia carried out an increased function of transporting a variety of chemotherapeutic drugs such as 5-FU, cisplatin, adriamycin, paclitaxel and gemcitabine, leading to decreased intracellular drug concentration and reduced cytotoxicity. Meanwhile, blocking the expression of HIF-1α in laryngeal cancer cells could abolish the upregulation of transport function induced by hypoxia. These results demonstrated that the function for drug transport which up-regulated by HIF-1α partly contributed to MDR in hypoxic laryngeal cancer cells. Further research is necessary to determine the exact mechanisms underlying the effect of HIF-1α on MDR in human laryngeal cancer.

In conclusion, our study demonstrates that HIF-1α may play a key role in mediating MDR in laryngeal cancer cells under hypoxic conditions. Meanwhile, our data indicate that two possible mechanisms for HIF-1α contributing to MDR are the resistance to drug-induced apoptosis and the decrease of intracellular drug concentration in vitro. All data may be of some interest for reversing MDR of human laryngeal cancer based on HIF-1α-dependent mechanisms.

Acknowledgements

We greatly appreciate Prof. Jin-ke Cheng and Miss Yan-qiong Zou, Morphology and Cell Chemistry Laboratory of Shanghai Jiao Tong University School of Medicine, for technical assistance. This work was granted by the Shanghai Science and Technology Development Fund (No. 09411951000), China.

References

Chou CW, Wang CC, Wu CP, et al (2012). Tumor cycling hypoxia induces chemoresistance in glioblastoma multiforme by upregulating the expression and function of ABCB1. *Neuro Oncol*, **14**, 1227-38.

Chu EA, Kim YJ (2008). Laryngeal cancer: diagnosis and preoperative work-up. *Otolaryngol Clin North Am*, **41**, 673-95.

Huang L, Ao Q, Zhang Q, et al (2010). Hypoxia induced paclitaxel resistance in human ovarian cancers via hypoxia-inducible factor 1alpha. *J Cancer Res Clin Oncol*, **136**, 447-56.

Huang S, Peng L, Tang Y, et al (2013). Hypoxia of PC-3 prostate

cancer cells enhances migration and vasculogenesis in vitro of bone marrow-derived endothelial progenitor cells by secretion of cytokines. *Oncol Rep*, **29**, 2369-77.
Jing SW, Wang YD, Kuroda M, et al (2012). HIF-1α contributes to hypoxia-induced invasion and metastasis of esophageal carcinoma via inhibiting E-cadherin and promoting MMP-2 expression. *Acta Med Okayama*, **66**, 399-407.
Li DW, Zhou L, Jin B, et al (2013). Expression and significance of hypoxia-inducible factor-1α and survivin in laryngeal carcinoma tissue and cells. *Otolaryngol Head Neck Surg*, **148**, 75-81.
Li L, Jiang AC, Dong P, et al (2007). The characteristics of Hep-2 cell with multiple drug resistance induced by Taxol. *Otolaryngol Head Neck Surg*, **137**, 659-64.
Liu L, Ning X, Sun L, et al (2008). Hypoxia-inducible factor-1 alpha contributes to hypoxia-induced chemoresistance in gastric cancer. *Cancer Sci*, **99**, 121-8.
Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*, **25**, 402-8.
Li Y, Ye D (2010). Cancer therapy by targeting hypoxia-inducible factor-1. *Curr Cancer Drug Targets*, **10**, 782-96.
O'Donnell JL, Joyce MR, Shannon AM, et al (2006). Oncological implications of hypoxia inducible factor-1alpha (HIF-1alpha) expression. *Cancer Treat Rev*, **32**, 407-16.
Park SY, Jang WJ, Yi EY, et al (2010). Melatonin suppresses tumor angiogenesis by inhibiting HIF-1alpha stabilization under hypoxia. *J Pineal Res*, **48**, 178-84.
Schrijvers ML, van der Laan BF, de Bock GH (2008). Overexpression of intrinsic hypoxia markers HIF1alpha and CA-IX predict for local recurrence in stage T1-T2 glotticlaryngeal carcinoma treated with radiotherapy. *Int J Radiat Oncol Biol Phys*, **72**, 161-9.
Sowter HM, Raval RR, Moore JW (2003). Predominant role of hypoxia-inducible transcription factor (Hif)-1alpha versus Hif-2alpha in regulation of the transcriptional response to hypoxia. *Cancer Res*, **63**, 6130-4.
Tak E, Lee S, Lee J, et al (2011). Human carbonyl reductase 1 upregulated by hypoxia renders resistance to apoptosis in hepatocellular carcinoma cells. *J Hepatol*, **54**, 328-39.
Thews O, Nowak M, Sauvant C, Gekle M (2011). Hypoxia-induced extracellular acidosis increases p-glycoprotein activity and chemoresistance in tumors in vivo via p38 signaling pathway. *Adv Exp Med Biol*, **701**, 115-22.
Wang GL, Jiang BH, Rue EA, Semenza GL (1995). Hypoxia-inducible factor 1 is a basic-helix-loop-helix-PAS heterodimer regulated by cellular O2 tension. *Proc Natl Acad Sci U S A*, **92**, 5510-4.
Wohlkoenig C, Leithner K, Deutsch A, et al (2011). Hypoxia-induced cisplatin resistance is reversible and growth rate independent in lung cancer cells. *Cancer Lett*, **308**, 134-43.
Wu XH, Lu YF, Hu XD, et al (2010). Expression of hypoxia inducible factor-1α and its significance in laryngeal carcinoma. *J Int Med Res*, **38**, 2040-6.
Yoshiba S, Ito D, Nagumo T, et al (2009). Hypoxia induces resistance to 5-fluorouracil in oral cancer cells via G(1) phase cell cycle arrest. *Oral Oncol*, **45**, 109-15.
Zhang L, Huang G, Li X, et al (2013). Hypoxia induces epithelial-mesenchymal transition via activation of SNAI1 by hypoxia-inducible factor -1α in hepatocellular carcinoma. *BMC Cancer*, **13**, 108.
Zhu H, Chen XP, Luo SF, et al (2005). Involvement of hypoxia-inducible factor-1-alpha in multidrug resistance induced by hypoxia in HepG2 cells. *J Exp Clin Cancer Res*, **24**, 565-74.