

Association between expression of HOTAIR and invasiveness of gliomas, and its predictive value

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Abstract

Background. Hox transcript antisense intergenic RNA (HOTAIR) is upregulated and associated with a poor prognosis in many cancer types. Besides, it is involved in the invasion and metastasis of non-small-cell lung cancer and nasopharyngeal carcinoma.

Objectives. The aim of this study was to investigate the association between the expression of HOTAIR and the grades of gliomas, and to explore its possible mechanism, as well as to evaluate the value of HOTAIR applied in predicting the grades of gliomas.

Material and methods. A total of 123 patients undergoing glioma surgeries were enrolled. Patients with grade I and grade II–IV tumors were regarded as the control group (n = 36) and the case group (n = 87), respectively. The expression of HOTAIR, matrix metalloproteinase 7 (MMP-7), matrix metalloproteinase 9 (MMP-9), and vascular endothelial growth factor (VEGF) was detected with quantitative reverse transcription-polymerase chain reaction (qRT-PCR) in glioma tissues and then compared between grade I and grades II–IV. The correlation between the relative expression of HOTAIR and that of MMP-7, MMP-9 and VEGF was analyzed. Multivariate analysis was performed to identify independent risk factors. Receiver operating characteristic (ROC) curve was employed to evaluate the predictive value.

Results. The relative expression of HOTAIR, MMP-7, MMP-9, and VEGF was lower in glioma tissues of grade I than in the case of grades II–IV, and the relative expression of HOTAIR was positively correlated with the relative expression of MMP-7, MMP-9 and VEGF. Multivariate analysis showed that the relative expression of HOTAIR was independently associated with the grades of gliomas, but the relative expression of MMP-7, MMP-9 and VEGF was not. Besides, multivariate analysis showed that the expression level of HOTAIR >0.40 was an independent risk factor for grades II–IV after classifying the relative expression of HOTAIR, and ROC analysis showed that the expression level of HOTAIR >0.40 had a moderate value when applied in predicting grades II–IV.

Conclusions. Hox transcript antisense intergenic RNA might promote the invasion of gliomas through upregulating the expression of MMP-7, MMP-9 and VEGF, and the expression level of HOTAIR >0.40 had a moderate value when applied in predicting grades II–IV.

Key words: gliomas, hox transcript antisense intergenic RNA, grades, predictive value, invasiveness

Introduction

As the most common primary tumors in the central nervous system among adults,^{1,2} gliomas are classified into 4 grades that reflect the degree of invasiveness/malignancy according to the current 2016 World Health Organization (WHO) Classification of Tumours of the Central Nervous System.³ Grade I refers to benign and more circumscribed tumors with low proliferative potential. Grade II–IV tumors are malignant and diffusely infiltrative with increased cellular abnormalities, and are referred to as diffuse gliomas because of the characteristic of extensive, diffuse infiltration of glioma cells into the brain tissue.⁴

Hox transcript antisense intergenic RNA (HOTAIR) belongs to long non-coding RNAs (lncRNAs). Many studies have indicated that lncRNAs can be applied in predicting the prognosis in various cancer types.^{5–8} Hox transcript antisense intergenic RNA is the first lncRNA discovered to have a trans-repressive role.^{9,10} It is upregulated and associated with a poor prognosis in many cancer types.^{11–15} Apart from that, it is involved in the invasion and metastasis of non-small-cell lung cancer and nasopharyngeal carcinoma through modulating the expression of matrix metalloproteinase 7 (MMP-7), matrix metalloproteinase 9 (MMP-9) and vascular endothelial growth factor (VEGF).^{16,17} In this paper, the association between the relative expression of HOTAIR and the grades of gliomas was analyzed, and the possible mechanism was explored; moreover, the value of HOTAIR applied in predicting the grades of gliomas was evaluated. The aim of the study was to determine the potential of HOTAIR as a biomarker of invasiveness for gliomas.

Material and methods

Patients

A total of 123 patients undergoing glioma surgery were enrolled in Heze Municipal Hospital, China, from January 2013 to January 2017. There were 70 males and 53 females among them, and their average age was 44.76 ± 15.82 years. A definite diagnosis of gliomas was made through a histopathologic examination in all patients. Neither radiotherapy nor chemotherapy were performed before operation. According to the current 2016 WHO Classification of Tumours of the Central Nervous System, grades I, II, III, and IV were found in 36, 32, 33, and 22 patients, respectively. The patients with grade I and grade II–IV tumors were regarded as the control group ($n = 36$) and the case group ($n = 87$), respectively. Immediately after excision, glioma tissue samples were placed in liquid nitrogen for 10 min, and then stored in a refrigerator at a temperature of -80°C . This study

received the approval of the ethics committee of Heze Municipal Hospital (approval No. hz201216039). All patients provided informed consent.

RNA extraction and quantitative reverse transcription-polymerase chain reaction

The total RNA from glioma tissues was extracted with the TRIzolTM reagent (Ambion, Cat. No. 15596-026; Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. The complementary DNA (cDNA) was synthesized with the First Strand cDNA Synthesis kit (Novagen, Cat. No. 69001-3; Merck & Co., Inc., Kenilworth, USA). The primer sequences for HOTAIR, MMP-7, MMP-9 and VEGF were 5'-GAGAACGCTGGAAAAACCTG-3' (forward) and 5'-GTCAGAAAATGCTTCCCCAA-3' (reverse), 5'-TACAGGATCATTTGGCTACACACC-3' (forward) and 5'-GGTCACATCGCTCCAGACT-3' (reverse), 5'-TGTACCGCTATGGTTACTACTCG-3' (forward) and 5'-GGCAGGGACAGTTGCTTCT-3' (reverse), and 5'-AGGGCAGAATCATCAGCAAGT-3' (forward) and 5'-AGGGTCTCGATTGGATGGCA-3' (reverse), respectively. *GADPH* was regarded as the reference gene (forward primer: 5'-AGGTCCACCACTGACACGTT-3'; and reverse primer: 5'-GCCTCAAGATCATCAGCAAT-3'). The amplification conditions were as follows: 94°C for 3 min; 40 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min; and 72°C for 7 min. The quantitative reverse transcription-polymerase chain reaction (qRT-PCR), data acquisition and analysis were performed with the ABI 7500 system (Life Technologies, Grand Island, USA). The relative expression of target genes was evaluated with the $2^{-\Delta\Delta\text{Ct}}$ method.

Statistical analysis

Statistical analysis was carried out using the IBM SPSS Statistics v. 21.0 for Windows (IBM Inc., Armonk, USA), and significance was set at $p < 0.05$. The distribution of data was determined with the Kolmogorov–Smirnov test. Normally distributed data was expressed as mean \pm standard deviation (SD) and compared with Student's t-test between glioma grade I and grades II–IV. Simple linear correlation analysis was employed to analyze the correlation between normally distributed data. In order to analyze the association between the expression levels of HOTAIR and the grades of gliomas, the relative expression of HOTAIR was classified into 4 levels based on the quartiles of grade I (level 1: $\leq\text{Q1}$; level 2: $>\text{Q1}$ and $\leq\text{Q2}$; level 3: $>\text{Q2}$ and $\leq\text{Q3}$; level 4: $>\text{Q3}$). Univariate analysis was performed with a simple χ^2 test. Multivariate analysis was then performed with a backward stepwise logistic regression model. Receiver operating characteristic (ROC) curve was employed to evaluate the predictive value of independent risk factors.

Results

Relative expression of HOTAIR

The relative expression of HOTAIR in glioma tissues of grade I (n = 36) and grades II–IV (n = 87) was 0.41 ± 0.20 and 0.80 ± 0.31, respectively. The difference was statistically significant (p < 0.001).

Relative expression of MMP-7, MMP-9 and VEGF

The relative expression of MMP-7, MMP-9 and VEGF in glioma tissues of grade I was 0.35 ± 0.17, 0.31 ± 0.19 and 0.39 ± 0.21, respectively, all of which were significantly lower compared to grades II–IV (0.79 ± 0.26, 0.70 ± 0.25 and 0.83 ± 0.35, respectively) (all p < 0.001).

Simple linear correlation analysis between relative expression of HOTAIR and relative expression of MMP-7, MMP-9 and VEGF

Simple linear correlation analysis showed that the relative expression of HOTAIR was positively correlated with the relative expression of MMP-7, MMP-9 and VEGF (r = 0.782, 0.791 and 0.778, respectively; all p < 0.05) in all 123 glioma patients.

Association between relative expression of HOTAIR, MMP-7, MMP-9 and VEGF, and grades of gliomas

Multivariate analysis showed that the relative expression of HOTAIR was independently associated with the grades

of gliomas after adjusting sex and age, but the relative expression of MMP-7, MMP-9 and VEGF was not independently associated with the grades of gliomas (Table 1). High expression of HOTAIR was an independent risk factor for grades II–IV.

Association between expression levels of HOTAIR and grades of gliomas

The classifying and univariate analysis results are shown in Table 2. Multivariate analysis showed that the expression level of HOTAIR >Q2 (0.40) was an independent risk factor for grades II–IV after adjusting sex and age (Table 3). The odds ratios (ORs) of level 3 and 4 were 5.061 and 9.016, respectively.

Predictive value of HOTAIR expression level

The results of the expression level of HOTAIR >Q2 (0.40) applied in predicting grades II–IV are shown in Table 4. The area under ROC curve (AUC) was 0.716 (standard error: 0.056; p < 0.001), which indicated that the expression level of HOTAIR >Q2 (0.40) had a moderate value when applied in predicting grades II–IV.

Discussion

Since HOTAIR is firstly discovered in human fibroblasts,¹⁸ many studies have reported that HOTAIR is significantly upregulated in many cancer types and play an important role in their development. For gliomas, HOTAIR can act as a prognostic factor for the survival

Table 1. Association between relative expression of HOTAIR, MMP-7, MMP-9 and VEGF, and grades of gliomas after adjusting sex and age

Genes	Regression coefficient	Standard error	Wald	OR	95% CI	p-value
HOTAIR	0.421	0.184	6.597	4.372	1.286–7.958	0.029
MMP-7	0.283	0.122	1.674	1.984	0.783–4.141	0.256
MMP-9	0.276	0.117	1.582	1.886	0.754–3.908	0.275
VEGF	0.291	0.124	1.785	2.094	0.791–4.247	0.244

HOTAIR – hox transcript antisense intergenic RNA; MMP-7 – matrix metalloproteinase-7; MMP-9 – matrix metalloproteinase-9; VEGF – vascular endothelial growth factor; OR – odds ratio; CI – confidence interval; Wald – the Wald χ^2 test. Grading of gliomas was regarded as the dependent variable (binary variable: grade II–IV was assigned to 1, and grade I to 0), and the relative expression of HOTAIR, MMP-7, MMP-9, and VEGF (quantitative variables), age (quantitative variable) and sex (binary variable) were regarded as the independent variables.

Table 2. Classifying and univariate analysis (simple χ^2 test) results of relative expression of HOTAIR

Relative expression of HOTAIR	Grades II–IV (n = 87)	Grade I (n = 36)	χ^2	p-value
Level 1: ≤Q1 (0.19)	2	9	32.379	<0.001
Level 2: >Q1 and ≤Q2 (0.40)	4	9		
Level 3: >Q2 and ≤Q3 (0.58)	19	9		
Level 4: >Q3	62	9		

Q1–Q3 – quartiles of grade I.

Table 3. Association between expression levels of HOTAIR and grades of gliomas after adjusting sex and age

Expression levels of HOTAIR	Regression coefficient	Standard error	Wald	OR	95% CI	p-value
Level 1	–	–	7.325	–	–	0.009
Level 2	0.412	0.283	1.584	1.575	0.332–3.658	0.274
Level 3	0.476	0.315	6.278	5.061	1.158–7.029	0.032
Level 4	0.511	0.367	18.213	9.016	1.392–12.164	<0.001

Grading of gliomas was regarded as the dependent variable (binary variable: grade II–IV was assigned to 1, and grade I to 0), and the relative expression of HOTAIR (rank variable), age (quantitative variable) and sex (binary variable) were regarded as the independent variables. Level 1 was regarded as the reference.

Table 4. Results of the expression level of HOTAIR >Q2 (0.40) applied in predicting grade II–IV

Cut-off value	Predicting criterion	Grade II–IV (n = 87)	Grade I (n = 36)
Q2 (0.40)	>0.40 (positive)	81	18
	≤0.40 (negative)	6	18

of glioma patients and a biomarker for the identification of glioma molecular subtypes.¹¹ In vitro, HOTAIR is associated with the progression of the cell cycle of glioma cells. Downregulation of HOTAIR can inhibit the proliferation, invasion and migration of tumor cells, and meanwhile promote the apoptosis of glioma cells.^{2,19} In our study, the relative expression of HOTAIR was lower in glioma tissues of grade I than in the case of grades II–IV, and multivariate analysis showed that high expression of HOTAIR (>0.40) was an independent risk factor for grades II–IV. These results suggested that HOTAIR was associated with the grades of gliomas and could act as a biomarker reflecting the invasiveness of gliomas.

Hox transcript antisense intergenic RNA is involved in the Wnt/ β -catenin, Akt and p53 signaling pathways through the combination with polycomb repressive complex 2 (PRC2) in tumor cells,¹⁴ which may affect the invasion and migration of tumor cells.²⁰ The Wnt/ β -catenin signaling pathway plays an important role in the development of tumors.²¹ HOTAIR can activate the Wnt/ β -catenin signaling pathway through downregulating the expression of Wnt inhibitory factor 1 (WIF-1),²² which may reduce the degradation of β -catenin in the cytoplasm.²³ β -catenin can upregulate the expression of VEGF, MMP-7 and cyclinD1 as a transcriptional factor after the entry into the nucleus. Vascular endothelial growth factor, MMP-7 and cyclinD1 may induce the formation of tumor vessels, and promote the invasion and migration of tumor cells.

As one of important tumor suppressor genes in the Akt signaling pathway, the phosphatase and tensin homology deleted on chromosome 10 (PTEN) gene can inhibit the activation of the Akt signaling pathway. Hox transcript antisense intergenic RNA may silence PTEN through enhancing the promoter methylation of PTEN, which may lead to the activation of the Akt signaling pathway. The activation of the Akt signaling pathway can promote the invasion and migration of tumor cells, and meanwhile reduce

the apoptosis of tumor cells by upregulating the expression of MMP-9 and downregulating the expression of BAX and FOXO1.¹² Besides, HOTAIR may induce the formation of tumor vessels by upregulating the expression of VEGF and downregulating the expression of transforming growth factor β (TGF- β) via the p53 signaling pathway.²⁴


In this study, the relative expression of HOTAIR, MMP-7, MMP-9, and VEGF was lower in glioma tissues of grade I than in the case of grades II–IV, and the relative expression of HOTAIR was positively correlated with the relative expression of MMP-7, MMP-9 and VEGF. However, multivariate analysis showed that the relative expression of MMP-7, MMP-9 and VEGF was not independently associated with the grades of gliomas. These results suggested that high expression of MMP-7, MMP-9 and VEGF might be induced by HOTAIR in grade II–IV tumors. Therefore, HOTAIR might promote the invasion of gliomas through upregulating the expression of MMP-7, MMP-9 and VEGF. Next, the value of the expression level of HOTAIR >0.40 applied in predicting grades II–IV was evaluated with ROC curve. This result showed that the expression level of HOTAIR >0.40 had a moderate value when applied in predicting grades II–IV.


Conclusions

Hox transcript antisense intergenic RNA might promote the invasion of gliomas through upregulating the expression of MMP-7, MMP-9 and VEGF, and the expression level of HOTAIR >0.40 had a moderate value when applied in predicting grades II–IV.

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