CORRELATION BETWEEN HOTAIR GENE POLYMORPHISM AND RISK OF BLADDER CANCER AND ITS EXPRESSION AND SIGNIFICANCE IN BLADDER CANCER

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ABSTRACT

Objective: *To explore the intrinsic correlation between HOTAIR gene traits and the risk of bladder cancer, and to clarify the expression of HOTAIR gene in bladder cancer patients.*

Method: *The experiment was conducted in Beijing Luhe Hospital from August 2006 to April 2008. The blood of the bladder cancer patients and the normal population was collected. On the basis of the extraction of DNA, the genotypes were classified. The genotype frequency distribution of the SNPs (single nucleotide polymorphisms) in the HOTAIR gene in the patients with bladder cancer and the control group was further compared and analyzed. The correlation between the SNPs genotype and the risk of bladder cancer was discussed. Taking the bladder cancer normal tissue as the reference, the expression of HOTAIR RNA in bladder cancer tissue was measured by qRT-PCR test, and the effect of HOTAIR on the survival time of bladder cancer patients and the correlation of prognosis were determined.*

Results: *Compared with the reference, the rs874945 locus of the HOTAIR gene significantly affected the frequency of the distribution of GG, AG and AA genotypes. Referring to GG gene type, the risk degree of bladder cancer in AG/AA and AG genotypes reached significant level (P<0.05), 1.095 times and 1.113 times as much as GG genotype, indicating that A allele increased the risk of bladder cancer to a certain extent, and there is a strong relationship between the two parts. Compared with normal tissue, the relative expression of HOTAIR in the bladder cancer tissue was significantly increased. The analysis of the expression of HOTAIR and the pathological related indicators showed that the stage and classification of the tumor were the main factors leading to the high expression of HOTAIR gene (P<0.05), and the survival rate of bladder cancer patients with low expression of HOTAIR in five years was significantly improved (P<0.05).*

Conclusion: *there is a significant correlation between the rs874945 locus in HOTAIR and the risk of bladder cancer, and the low expression of HOTAIR gene can effectively improve the survival rate of bladder cancer patients.*

Keywords: *HOTAIR gene, gene polymorphism, bladder cancer, onset risk.*

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Introduction

At present, the tumor seriously threatens and damages the human body health system. For malignant tumors, the prevention and control is mainly achieved through surgical removal and chemotherapy[(1)]. Worldwide, bladder cancer is a common tumor in the genitourinary system, which causes serious damage to human health[(2)]. With the increase of population in China, the proportion of bladder cancer patients is increasing year by year, which leads to the increasing incidence and mortality of bladder cancer and causes the people to pay wide attention to bladder cancer[(3)].

Clinical studies show that in bladder cancer, the transitional cell carcinoma has a high degree of instability and high recidivity, and chemotherapy cannot make it effectively treated. The effect of chemotherapy is usually insensitive, the anti-tumor drug resistance is easily caused, and the therapeutic effect is poor[(4)]. Compared with other malignant tumors, bladder cancer patients have a relatively

low mortality and long survival time after surgical excision, but most of the bladder cancer patients have to undergo multiple operations, and the more serious patients need to remove the whole bladder, which greatly reduces the living quality of the patients in the later period of life[5]. Because of the sharp rise in the incidence and mortality of bladder cancer, it seriously endangers the health of the people. The expensive operation costs cause great economic pressure, which brings great fear to the masses, and it is important to study the mechanism of bladder cancer. Therefore, it is of great theoretical significance and research value to study the pathogenesis of bladder cancer and to explore the mechanism of its inhibition and prevention, which can effectively control the risk of the disease in the future and reduce the incidence of bladder cancer.

It is found that the HOTAIR gene has a state of abnormal expression in most of the tumors. The degree of relative expression of the gene is closely related to the formation and development of the tumor. Therefore, the HOTAIR gene can be used for the measurement of the risk of cancer and reference and basis for the recurrence and metastasis of the tumor after treatment[6]. At present, by scientific research, the biological functions of HOTAIR cannot be fully understood, but a small number of studies show that HOTAIR's biological information regulation is reflected at the genetic level. The HOTAIR gene can rebuild the carrier on the basis of the recruitment of chromatin and immobilize the complex on the HOXD site, which further makes the HOXD site achieve epigenetic silence and the gene expression inhibited. This shows that HOXD plays a key regulatory role in the modification of histone and the correct expression of genes[7]. Gupta et al. suggested that the HOTAIR gene could bind the multiple comb protein inhibitory complex, which could participate in the recombination of chromatin in the nucleus and silence some of the related tumor suppressor genes to further enhance the metastasis of the tumor[8]. When the relative expression level of HATOIR in bladder cancer tissues was measured, Yan et al. pointed out that the expression level of HOTAIR was closely related to bladder cancer. When the relative expression level of HOTAIR gene was high, the probability of the recurrence of the bladder cancer patients could be significantly improved. The reason for the biological mechanism might be that 101NR greatly inhibited the expression of WIF-1 gene[9]. Similarly, some scholars focused on genetic variation of lncRNAs gene. Zhou et al. proposed that the rs2839698 locus of lncRNAHl9 gene had a significant correlation with the risk of bladder cancer, which could effectively reduce the onset risk[10]. Xue et al. studied lncRNA HOTAIR genes and found that the genetic variation of the gene was associated with the risk of colorectal cancer. They also pointed out that the rs7958904 locus in the HOTAIR gene had a certain degree of decrease in the incidence of low colorectal cancer. Moreover, they further carried out a cell experiment, and found that, compared with the G allele; the C allele of rs7958904 was more potent in the proliferation of cancer cells in colorectal cancer[11]].

To sum up, the HOTAIR gene plays an important role in the biological research of tumor, but there are few reports about the relationship between HOTAIR gene and bladder cancer. The relationship between the SNPs locus of HOTAIR gene and the risk of bladder cancer is studied. Then, the relationship between the genotype distribution and the risk of bladder cancer and the mechanism of bladder cancer are identified, and the expression and significance of HOTAIR in bladder cancer are explored.

Materials and methods

Experimental materials

The test was conducted in Beijing Luhe Hospital from August 2006 to April 2008. 80 patients with bladder cancer were selected as the control group and 80 of the normal population were selected as the control group. Among them, the structure of the two groups was the same, 70 men and 10 women, 40 cases older than 65 years old, and 40 cases between the age of 45~65 years. And the fasting peripheral venous blood of the two test group was extracted. After mixing, plasma, red blood cells and leukocyte layer were separated, for cryopreservation at -20 DEG C fridge. The cancer tissue and normal tissue adjacent to the cancer of the bladder cancer patients were scrubbed with aseptic saline and quickly placed in the liquid nitrogen. After the treatment, it was immediately placed into the -80 DEG C fridge for use, and the control group was normal bladder mucosa 3cm from the edge of the tumor. All the subjects in the study have signed informed consent and have been approved by the ethics committee of the Beijing Luhe Hospital.

Experimental methods

Design and synthesis of primers

All the test steps and tests were carried out on the ice. 10μL SYBR PremixExTaqII (Bao RI Medical Biotechnology Co., Ltd., Beijing, China), 20μL RT-PCR reaction system, 2μL cDNA with concentration of 500ng/20μL, 0.8μL primer with concentration of 10pmol/L, 6μL DEPC-treated water, and 0.4μL ROX ReferenceDye (50*) (Bao RI Medical Biotechnology Co., Ltd., Beijing, China) were mixed. The temperature keeps at about 95 DEG C and continues for 30s, and circulates 40 times after the pre-denaturation. According to the following conditions: the temperature is denatured at 95 DEG C, which continues for 5s; the temperature is decreased to 60 DEG C and annealed, which lasts for 30s; the temperature is set at 95 DEG C, and the time is 30s; the temperature is set at 95 DEG C, and the time is 3s; the temperature is set at 60 DEG C for 30s. Then, the reagent was fully mixed and centrifuged by centrifuge (Form Company, Germany), and the labeled pipes were conducted with fluorescence quantitative PCR (Polymerase Chain Reaction).

Screening and confirmation of the tag SNPs locus of HOTAIR

The location information of HOTAIR gene is searched combined with lncRNA database NCBI database. According to the Refseq gene library of NCBI, the physical location of the HOTAIR gene in the genome was determined. By using the Tagger pairwise algorithm, two SNPs sites to be studied were selected: rs874945 and rs7958904.

RNA extraction experiment

50mg sample tissues were placed inside the grinding tube, added with the beads of RNA enzyme and 1mL Trizol (Invitrogen Company, America), and reacted for 1min on the homogenizer with a frequency of 60HZ. After the reaction was completed, the homogenate samples were placed at 25 DEG C and static for 5min. 1.5mL homogenate samples were taken and placed in the centrifuge tube, and homogenate was centrifuged at 4 DEG C centrifuge (Suzhou Purification Equipment Company, Jiangsu, China) 12000g for 10min. A certain amount of supernatant was extracted and placed in the new centrifuge tube, and 1mL Trizol and 0.2 mL chloroform were added, respectively, being static for 10min after uniformly mixed. Then, it was centrifuged with 12000g at 4 DEG C centrifuge for 15min. In the upper system, namely RNA layer, the volume was about 60% of Trizol used. The upper liquid was transferred to the centrifuge tube without nuclease, and 0.5mL isopropanol was added and placed at 25 DEG C for 10min. Then, it was centrifuged with 10000g at 4 DEG C for 10min. After centrifugation, the RNA precipitate was a white floc attached to the wall of the centrifuge tube. After the supernatant was removed, 1mL 75% nuclease free ethanol was used for washing. The washing solution was centrifuged with 7500g at 4 DEG C for 5min and repeated two times. After settling the precipitate at 25 DEG C for 5min, the ethanol was dropped out. 25-200μL water that did not contain nuclease was added and the test qualified RNA was transferred to the refrigerator at -80 DEG C to be stored.

Total RNA reverse transcriptase synthesis of cRNA

The RevertAidTM first chain cDNA synthesis kit (Beijing Quanshijin Biotechnology Co., Ltd., Beijing, China) was used to reverse transcriptional synthesis of RNA. Firstly, the reaction system was constructed on the ice, including 2.0μL total RNA (1μg), 0.5μL Oligo (dT)18 primers, 0.5μL random six-polymer primers, and 9μL nucleic acid free high-pure water, and then incubated at 65 DEG C after mixing for 5min. 4.0μL 5X Reaction Buffer was added to the PCR reaction tube, and 1.0μL RiboLock™ RNA enzyme inhibitor with the concentration of 20u/μL, 2.0μL 10mM dNTP Mix and 1.0μL RevertAid™ M-MuLV reverse transcriptase with concentration of 200u/μL were added. After mixing the above liquids, they were centrifuged, placed in ABI7900 Realtime-PCR instrument (LFS-312W, Shanghai, China) and reacted at 25 DEG C for 5min. Subsequently, it was incubated at 42 DEG C for 60min and reacted 5min at the temperature of 70 DEG C and then terminated immediately, and the cRNA synthesized by reverse transcriptase was kept in the refrigerator at -20 DEG C.

Real-time fluorescence quantitative PCR detection of bladder cancer tissue and HOTAIRRNA expression in bladder cancer tissues

The 20uL reaction system was used for amplification. The cRNA template used the ultrapure water treated with 1μL DEPC as negative control. It was treated at 95 DEG C for 10min; the temperature was 95 DEG C and treated for 15s; the temperature was 60 DEG C and treated for 1min; and the 45 cycle was carried out according to the secondary reaction system.

Statistical method

The data were analyzed by SPSS18.0 software. The expression of HOTAIR in cancer tissues and adjacent tissues was expressed by the mean value of three tests. The relationship between the expression of HOTAIR and the prognosis of bladder cancer patients was analyzed by Kaplan-Meier survival analysis and Log-Rank test, and the level of the test was α=0.05.

Results

Relationship between frequency distribution of HOTAIR genotype and risk of bladder cancer

The 2 different SNPs loci of the HOTAIR gene were rs7958904 and rs874945, respectively. The frequency distribution of alleles of the genotype in the two groups of bladder cancer patients and the control group, respectively, was shown in Table 1.

SNP (single nucleotide polymorphisms)	Genotype	Cases (%)	Control (%)	OR (95% CI)	P
rs874945	G/G	61.9	66.7		
	A/G	35.3	29.8	1.113 (1.030,1.203)	0.006
	A/A	2.8	3.5	0.938 (0.787,1.118)	0.556
	A/A+ A/G	38.1	33.3	1.095 (1.017,1.179)	0.017
	G	68.3	93.2		
	A	31.7	6.8	1.050 (1.011,1.130)	0.042
rs7958904	G/G	85.6	87.2		
	A/G	12.3	10.2	1.095 (0.972,1.233)	0.119
	A/A	1.8	2.4	0.897 (0.731,1.101)	0.399
	A/A+ A/G	14.1	12.7	1.057 (0.951,1.175)	0.306
	G	78.7	91.4		
	A	21.3	8.6	1.026 (0.935,1.126)	0.589

Table. 1: Relationship between frequency distribution of HOTAIR genotype and risk of bladder cancer in two groups.

The allele distribution frequencies of rs7958904 locus in the bladder cancer group and the control group were not statistically significant (P>0.05), and there was no statistical difference between the various types of genetic models. However, in the rs874945 locus, the distribution frequencies of AG and AA/AG genotype were significantly different (P<0.05) in the two experimental groups compared with the frequency of GG genotype distribution. The risk of bladder cancer in individuals with AG/AA genotype was 1.095 times higher (95% CI=1.017~1.179, P=0.017) compared to the GG genotype, and the risk of bladder cancer in the AG gene was 1.113 times (95%CI=1.030, 1.203, P=0.006) higher than that of the GG genotype. The frequency of allele distribution also revealed the correlation in the risk of disease.

From Table 1, it was found that there was a clear correlation between the A allele and the risk of bladder cancer compared with G allele (OR=1.050, 95%CI1.011~1.1 30, P=0.042), indicating that A allele is a key factor leading to the risk of bladder cancer.

Expression of HOTAIR in bladder cancer and normal tissues

As shown in Figure 1, the relative expression level of the HOTAIR gene in the bladder cancer tissues and the normal tissues reaches a significant difference level, and the two have statistical significance. Among them, the expression level of the HOTAIR gene in the cancer tissues is high, but the expression level is low in the normal tissues, and the expression level in the cancer tissue is significantly improved for 147.4% than that in the normal tissue (P<0.05).

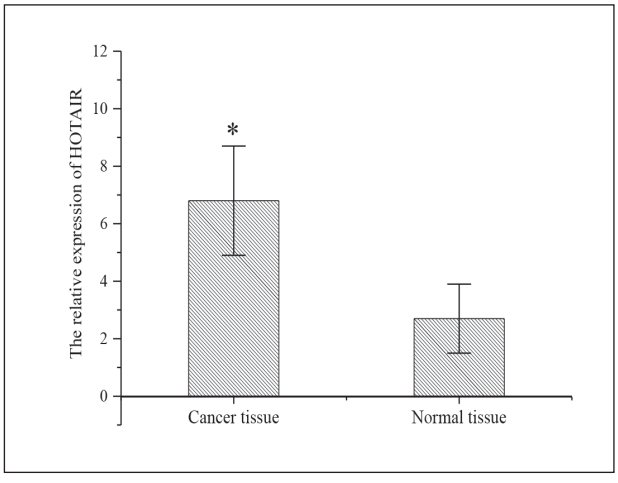

Fig 1: The relative expression of HOTAIR in cancer and normal tissues.

Relationship between HOTAIR expression and clinical pathological features in bladder cancer

From Table 2, it can be seen that the HOTAIR gene has different levels of high and low expression in the bladder cancer tissues, and the high level of HOTAIR has a significant correlation with the different stages and the levels of the tumor (P<0.001). However, the correlation with the age, gender and the size of the tumor does not reach a significant level. Therefore, the expression of HOTAIR gene is closely related to the development of this tumor.

Expression/n (%)					
Influencing factors	Category	HOTAIR		X^2	*P*
		high expression	Low expression		
Age	<65	58.5	41.5	50.254	0.671
	≥65	53.2	46.8		
Gender	Female	36.4	63.6	1.901	0.205
	Male	58.4	41.6		
The size of tumor (cm)	<3	59.5	40.5	0.369	0.664
	≥3	52.9	47.1		
Tumor staging	T_a~T_1	82.4	17.6	15.972	<0.001
	T_2~T_4	38.9	61.1		
Tumor grading	Low grade	87.5	12.5	11.213	0.001

Table. 2: Relationship between HOTAIR expression and clinical pathological features in bladder cancer.

Effect of HOTAIR expression on survival time of patients with bladder cancer

The effect of HOTAIR expression in cancer tissue on the 5-year survival rate of bladder cancer patients was further analyzed. As shown in Figure 2, 3, the survival time function of bladder cancer patients decreased gradually with time, and the survival rate of patients decreased rapidly from the third to fifth years. At the same time, the survival time of patients with lower HOTAIR expression in bladder cancer patients was generally higher than those with high expression of HOTAIR, and there was a significant statistical difference between the two (P<0.001).

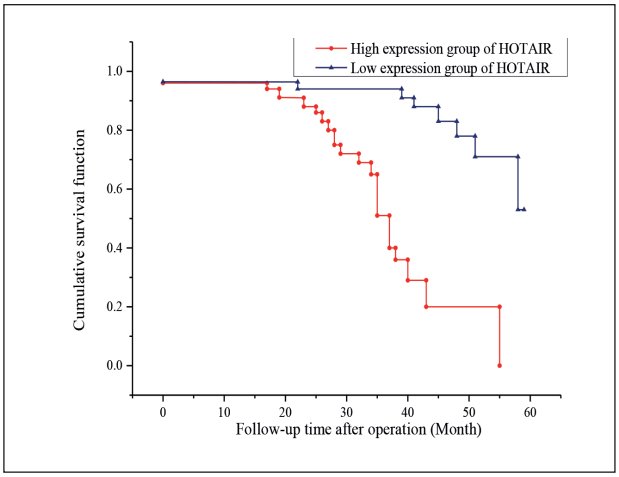

Fig 2: Survival rate of patients with different bladder cancers in HOTAIR expression group.

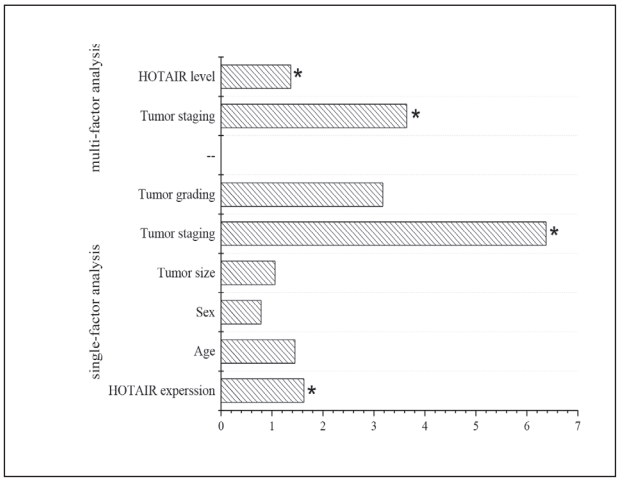

Fig 3: Univariate and multivariate analysis of predictors of bladder cancer survival.

The COX proportional risk regression analysis model was used to conduct the analysis of single factor and multifactor associated with the survival of bladder cancer patients. Single factor analysis showed that the stage of tumor and the expression level of HOTAIR gene in the cancer tissues were closely correlated with the survival rate of the patients, and the multifactor analysis showed that the expression level of HOTAIR in bladder cancer and tumor staging were also significantly correlated with survival rate. Therefore, the survival rate of bladder cancer patients is mainly affected by the level of HOTAIR gene expression within 5 years.

Discussion

In recent years, a large number of studies have shown that lncRNA has been involved in a large number of biological activities, such as regulating cell proliferation and differentiation, embry-

onic development, formation of organs, gene imprinting, participating in the regulation of genetic genotypes, reconstructing proteins and chromosomes, and modifying histone, which are mainly in the form of RNA. Zhou et al. studied and found that the rs2839698 locus in lncRNAHl9 gene could effectively control the risk of bladder cancer, and had a significant correlation with reducing the incidence of bladder cancer[10].

Jin et al. found a significant correlation between SNP rs3787016 locus and the increased risk of prostate cancer[12]. At the same time, the research on the genetic variation of lncRNA gene and its biological function has also become a hot topic of current research. Xue et al. explored lncRNA HOTAIR genes and found that the genetic variation of the gene was certainly associated with the risk of colorectal cancer. They also pointed out that the rs7958904 locus in the HOTAIR gene had a certain degree of decrease in the incidence of low colorectal cancer. They further carried out a cell experiment, and found that, compared with the G allele, the C allele of rs7958904 was more potent in the proliferation of cancer cells in colorectal cancer[11].

Two SNPs, namely rs7958904 and rs874945 were obtained through selecting the corresponding label SNPs within the HOTAIR gene range from the NCBI database. The blood of the bladder cancer patients and the normal population was collected. On the basis of the extraction of DNA, the genotypes were classified, and the genotype frequency distribution of SNPs in HOTAIR gene of the bladder cancer patients and the control population was further compared and analyzed. The correlation between SNPs genotype and the risk of bladder cancer was discussed. It was found that the allele distribution frequencies of rs7958904 locus in the bladder cancer group and the control group were not statistically significant ($P>0.05$), and there was no statistical difference in the genetic models of each type. However, in the rs874945 locus, the distribution frequency of AG and AA/AG genotype was significantly different in the two test groups compared with the GG genotype frequency ($P<0.05$). The risk of bladder cancer in the AG/AA genotype was 1.095 times higher than that of the GG genotype, and the risk for the AG genotype was increased by 1.1. 13 times.

The distribution frequency of allele also revealed the correlation in the risk of the disease. From Table 1, it was found that there was a significant correlation between the A allele and the risk of bladder cancer compared with the G allele, suggesting that the more the A alleles, the higher the risk of bladder cancer.

In the course of the formation and development of tumor, the abnormal regulation of gene expression plays a very important role. Generally speaking, the phenomenon of nucleotide transcription occurs generally in the autosomes of the human body. Based on this, a large number of research work has been put into lncRNA to make it associated with human diseases, to understand and analyze the expression of lncRNA in the disease, and to understand the mechanism of disease formation and development[13-14]. HOTAIR is one of the members of the lncRNAs gene system. Its location is defined as a fragment between the HOXC11 gene and the HOXC12 gene of the HOXC family on the human chromosome 12q13.13, and the transcriptional fragment is only a small length, only 2.2kb[15]. Wang et al. studied the breast cancer and found that the HOTAIR gene could achieve high levels of expression in the primary breast cancer and its metastasis, which greatly illustrated the close relationship between the HOTAIR gene and the development of breast cancer and the development of metastasis[16]. Kim et al. found that the expression of HOTAIR gene was obviously improved in most of the tumors, including liver cancer, gastrointestinal stromal tumor and pancreatic cancer. The higher the expression of HOTAIR was, the shorter the survival time of the patients with the disease was[17].

The expression of HOTAIR gene in the cancer tissues and normal tissues of patients with bladder cancer was detected by qRT-PCR. The results showed that the expression level of HOTAIR gene in the cancer tissues was high, but the expression level in normal tissues was low. The relationship between the expression of HOTAIR and the clinical pathological features in the bladder cancer tissues suggested that HOTAIR with high level of expression had a significant correlation with different stages and levels of tumor ($P<0.001$), but there was no more obvious correlation with age, gender and tumor size. The analysis of 5-year survival rate of bladder cancer patients with low and high level of HOTAIR expression showed that the survival time of patients with low HOTAIR expression in bladder cancer patients was generally longer than that of patients with high HOTAIR expression, and there was a statistically significant difference between the two. Combined with univariate and multivariate analysis, high-expression HOTAIR was a major risk factor for survival in patients with bladder cancer.

Conclusion

To sum up, the A allele of the rs874945 locus in the HOTAIR gene is a major factor for improving the risk of bladder cancer. HOTAIR has a high expression in the bladder cancer tissue. The high expression of HOTAIR is closely related to the pathological staging and grading of bladder cancer.

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