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Relationship between expression of Aurka and clinicopathological characteristics in gastric cancer patients

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ABSTRACT

The aim of the study was to clarify the role of Aurka in the process of formation and development of gastric cancer. The expression of Aurka in gastric cancer and corresponding non-cancerous gastric tissues of 52 gastric cancer patients was assessed with the real-time fluorescence quantitative polymerase chain reaction (RT-PCR) and immunohistochemistry. We also analyzed the relationship between the expression level and clinicopathological characteristics. Aurka gene and protein expression in gastric cancer tissues in both cases were significantly higher than in corresponding non-cancerous gastric tissues (both P < 0.01), but no significant relationship was found with clinicopathological parameters including tumor location, depth of invasion, differentiation, lymph node metastasis, stage, gender, age and carcinoembryonic antigen (CEA), and carbohydrate antigen 19-9 (CA19-9) level in peripheral blood preoperation of patients (P > 0.05, respectively). Furthermore, Aurka gene expression was markedly higher in gastric cancer tissues of *Helicobacter pylori* (HP)-positive patients than negative ones (P < 0.05). The result of the study showed that Aurka might play a significant role in the process of formation and development of gastric cancer as an oncogene. Its effect might be strengthened by HP infection.

KEYWORDS

Aurka; Clinicopathology; Gastric Cancer; *Helicobacter pylori*

1. INTRODUCTION

Gastric cancer, a kind of the most common malignant tumors worldwide, is the second leading cause of cancer death. It was estimated that there were about 989 thousand new gastric cancer cases and 738 thousand gastric cancer deaths globally one year [1]. Tumor cell infinite growth and invasion and metastasis are the most important characteristics of malignant tumors. Its diverse invasion ways and metastasis are the important causes of gastric cancer treatment failure and the high mortality rate. The occurrence as well as the development of gastric cancer is a polygenic, multifactorial and complex multi-stage evolution, involving a variety of oncogenes and tumor suppressor genes. Aurora Kinase A (Aurka) is one of the three serine/threonine kinases (A, B, C) that are evolutionarily conserved and regulate mitosis progression in various organisms. It controls centrosome maturation and separation, mitotic entry, spindle formation, and chromosome Alignment [2]. Gene overexpression or distortion can cause cancerous cells. Aurka was found in many tumors with high expression, eg: breast cancer [3], ovarian cancer [4], esophageal [5], gastric cancer [6]. Colon [7], and was closely associated with tumor development; therefore, to explore the relationship between gastric cancer and Aurka and its mechanisms

will help us provide a new target for the treatment. The expression of Aurka in gastric cancer and corresponding non-cancerous gastric tissues was detected with RT-PCR and INH. Finally, the relationship between its expression and clinicopathological characteristics of gastric cancer was analyzed.

2. MATERIALS AND METHODS

2.1. Patient Characteristics

After inform consent forms were signed, the specimens including gastric cancer and corresponding non-cancerous gastric tissues in general surgery of affiliated hospital of North Sichuan Medical College during April to July in 2013 were collected. All cases were diagnosed as gastric adenocarcinoma with pathological biopsy both preoperation and postoperation. They who had taken anti-HP drug or chemotherapy or radiotherapy lately were removed. The clinicopathological characteristics of patients are summarized in **Table 1**.

Table 1. Patient clinicopathological characteristics.

Characteristics		N = 52
Gender	Male	41
	Female	11
Age (years)	Mean	58.6 ± 11.7
	Range	31 ~ 79
Differentiation	Well	2
	Moderate	14
	Poor	36
Location of tumor	Upper	13
	Middle	14
	Lower	25
Depth of invasion*	T1	10
	T2	10
	T3	0
	T4	32
Lymph node metastasis*	N0	20
	N1	10
	N2	14
	N3	8
Stage*	I	15
	II	10
	III	24
	VI	3

*According to American Joint Committee on Cancer (2010).

2.2. Samples

All samples consisted of gastric cancer and corresponding non-cancerous gastric tissues were obtained intraoperation just after they were cut off. Then part were put into −80˚C refrigerator quickly used for total RNA extraction. The left were dipped in formalin and then embedded with paraffins for INH.

2.3. Detection of HP

Patients were arranged to take 14C-urea breath test to detect HP value preoperation. They whose HP values lower than 100 dpm were regarded as negative, and the others positive. Due to cardia complete obstruction or poor compliance, 11 patients did not accept ^{14}C-urea breath test.

2.4. RT-PCR

104 gastric cancer and corresponding non-cancerous gastric tissues were ground into fine powder with mortars and pestles in liquid nitrogen. Then total RNA was extracted with TRizol Reagent (Tiangen, China). Later the quality of total RNA was assessed with ultraviolet spectrophotometer (SHIMADZU, Japan) and agarose gel electrophoresis (agarose from Sigma, America; electrophoresis apparatus from BIO-RAD, America). cDNA was synthesized using reverse transcription kit (BioBRK, China) according to instruction. RT-PCR thermocycler (ABI, America) and kit (Takara, Japan) including Pre Mix, Dye and DNase/RNase free ddH2O were used for cDNA amplification. 1 μl cDNA, 10 μl Pre Mix, 2 μl Dye, forward and reverse primer both 0.6 μl (10 pmol/μl) and 5.8 μl DNase/RNase free ddH_2O were consisted in amplification system. Aurka and *β*-actin primers were synthesized by invitrogen company. The primer sequence, reaction condition and product size are all in **Table 2**. All samples were tested duplicately. After amplification finished, dissociation curve was analysed to identify the uniqueness of product. $2^{-\Delta CT}$ was used as relative expression value.

2.5. INH

Paraffins embeded 104 gastric cancer and corresponding non-cancerous gastric tissues were sectioned to 3 μm in thickness. After the slices were dipped in dimethylbenzene twice each for 10 min, they were put into 100% alcohol, 85% alcohol and 75% alcohol successively, and then washed with running water for deparaffinization. 3% H_2O_2 covered the whole tissues on slices for 20 min at room temperature and away from light in order to block endogenous peroxidase activity. Heat antigen retrieval was performed in 0.01 M citrate buffer (ph 6.0) at 95˚C for 20 min. Then slices were incubated with Rabbit anti-

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Table 2. Primer sequence, reaction condition and product size.

Primer name	Primer sequence	Product size (bp)	Reaction condition
Aurka	F GGAATATGCACCACTTGGAACA	108	1) 95˚C 30 sec 1 cycle
	R TAAGACAGGGCATTTGCCAAT		
β-actin	F GAGCTACGAGCTGCCTGAC		2) 95˚C 3sec
	R GTAGTTTCGTGGATGCCAC	120	60˚C 30 sec 40 cycles

Aurka monoclonal antibody (ABCAM, England) at a dilution of 1:150 (final concentration: 6.67 μg/ml) at 4˚C overnight. At the next morning the slices were incubated with common secondary antibody (ABCAM, England) at room temperature for 15 min. After colorated with diaminobenzidine and counterstained with hematoxylin, the reaction products were visible. All slices were assessed by two professors of pathology who had no knowledge of any clinicopathological characteristics of the patients. Immunohistochemical results were evaluated for intensity and staining frequency of nuclear and cytoplasmic components. The intensity of staining was graded 0 (negative), 1 (weak), 2 (moderate), and 3 (strong). The frequency was graded from 0 to 4 according to the percentage of positive cells as follows: 0, ≤3%; 1, ≤4% to 25%; 2, ≤26% to 50%; 3, ≤51% to 75%; 4, ≥75%. The products of multiplication of the intensity and frequency grades were then classified into an index core on a scale of 0 to 3: index score 0 = product of 0 (negative), index score 1 = products of 1 and 2 (weak), index score 2 = products of 3 and 4 (moderate), index score 3 = products of 6 through 12 (strong) [8].

2.6. Statistical Analysis

T-test or One-way analysis of variance were used for PCR data statistics, and Chi-square test was used for INH results. $P < 0.05$ was considered to have statistical significance.

3. RESULTS

3.1. Expression of Aurka Gene by RT-PCR

3.1.1. Expression of Aurka Gene in Gastric Cancer and Corresponding Non-cancerous Gastric Tissues

The mean ΔCT value of *Aurka* in gastric cancer and corresponding non-cancerous gastric tissues were 6.63 ± 1.85; 17.20 ± 1.59 ($t = -38.58$, $p < 0.01$) **Figure 1**, The difference was of statistical siginificance. The amplification curve and dissociation curve are in **Figure 2**.

3.1.2. Relationship between Aurka Gene Expression and HP of Gastric Cancer Patients

The mean ΔCT value of *Aurka* in gastric cancer tissues of HP positive and negative patients were 5.70 ± 1.86 and 7.88 ± 1.73 respectively. The variation was siginificant ($t = -3.74$, $P < 0.05$; **Table 3**).

3.1.3. Relationship between Aurka Gene Expression and Clinicopathological Characteristics of Gastric Cancer Patient

The CEA data of 3 patients and CA19-9 of 2 patients were lost, so they were removed when made those two statistics analysis. No siginificant relationship was found between *Aurka* and clinicopathological characteristics, including gender, age, tumor's location, depth of invasion, differentiation, lymph node metastasis, stage and CEA, CA19-9 level in peripheral blood preoperation (all $P > 0.05$; **Table 4**).

3.2. Expression of Aurka Protein by INH

The difference between positive rate of *Aurka* protein in gastric cancer and corresponding non-cancerous gastric tissues, 94.2% (49/52) and 15.4% (8/52), was significant in statistics ($P < 0.01$). See **Figures 3** and **4**.

4. DISCUSSION

The human Aurka gene located in chromosome band 20q13.2, full-length cDNA contains 1212 kb open reading frame encodes a molecular weight of 46 kDa, 403amino acid serine/threonine kinases. Aurka are positioned at the center body From copy to the end of the mitosis [9]. In addition, Aurka also appeared in the microtubule region near the center of the body [10] can regulate centrosome separation, maturation and spindle assembly, play an important role in cell cycle G2/M phase transition and the detection point .In 1998, Human Aurka Gene was first cloned from the colon while it was determined to be oncogene, because overexpression of Aurka can tumor born mice [7]. Aurka also significantly upregulated in a variety of other tumor tissues and various cancer cell lines due to gene amplification and enhanced translation [11,12]. Sen [13] first discovered Aurka overexpression in primary mammary, subsequent study has also found Aurka certain extent overexpressed in the human breast, ovary, pancreas, stomach tumors. Tumor formation is the cumulative result of a series of genetic mutations, there is prevalence phenomenon of genomic instability in a variety of tumor cells [14] just as gastric cancer. The mutation of Cancer-related genes, including oncogenes, tumor suppressor genes and genetic stability (e.g., DNA repair gene) can cause the genome

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instability [15]. According to the known cytological features of Aurka, we speculate Aurka may be involved in tumor formation through two functions: First, inhibit the cytokinesis split in the process of mitosis which made the instability of genomic [16,17]. Second, inhibit the checkpoint of cell cycle, helping instability cells of the genomic continue to replicate, continue to enter mitosis, leading to cell malignant proliferation.

In esophageal, gastric metaplasia, intestinal metaplasia, low-grade intraepithelial neoplasia, high-grade intraepithelial neoplasia, Pap, esophageal adenocarcinoma, the expression level of Aurka in esophageal mucosa is gradually increasing [18],which showing its expression in esophageal carcinogenesis through the whole process and plays an important role in the incidence and the development process of esophageal cancer, And yet in this regard reported in gastric cancer, if we can find some of its relationship with the pathological features of gastric cancer through research, then the next preoperative endoscopic biopsy of the tumor preliminary assessment will help to guide clinical treatment. However, very few similar studies, whether there has relationship between the expression of Aurka and pathological features of gastric cancer and HP values are related, there is no research in this area yet at home and abroad; Also there is no reports at home and abroad whether there has relationship between their peripheral blood CEA and CA19-9 levels in patients with gastric cancer as well as with gender, age, worthing further study. In this study, RT-PCR and immunohistochemical methods are used to detect the levels of Aurka in gastric cancer and normal tissues from gene and protein, respectively, combined with clinical data and tumor pathological characteristics of patients were compared to clarify the above-described problems.

Our experiment results, both Aurka gene and protein expressing in gastric cancer tissues were significantly higher than in corresponding non-cancerous tissues, con-

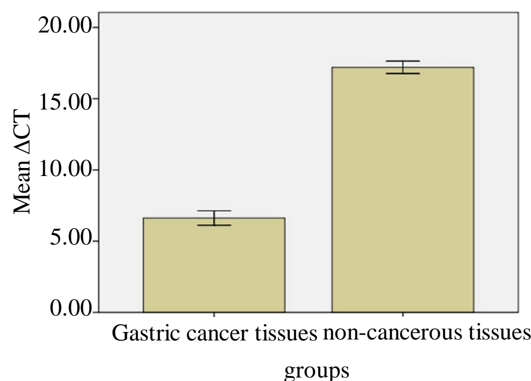

Figure 1. The mean ΔCT value of *Aurka* in gastric cancer and corresponding non-cancerous gastric tissues.

Table 3. The expression of aurka gene in gastric cancer tissues in HP.

Group	N	Aurka mRNA	P
		Mean ΔCT ± S	
+	22	5.70 ± 1.86	0.001
−	19	7.88 ± 1.73	

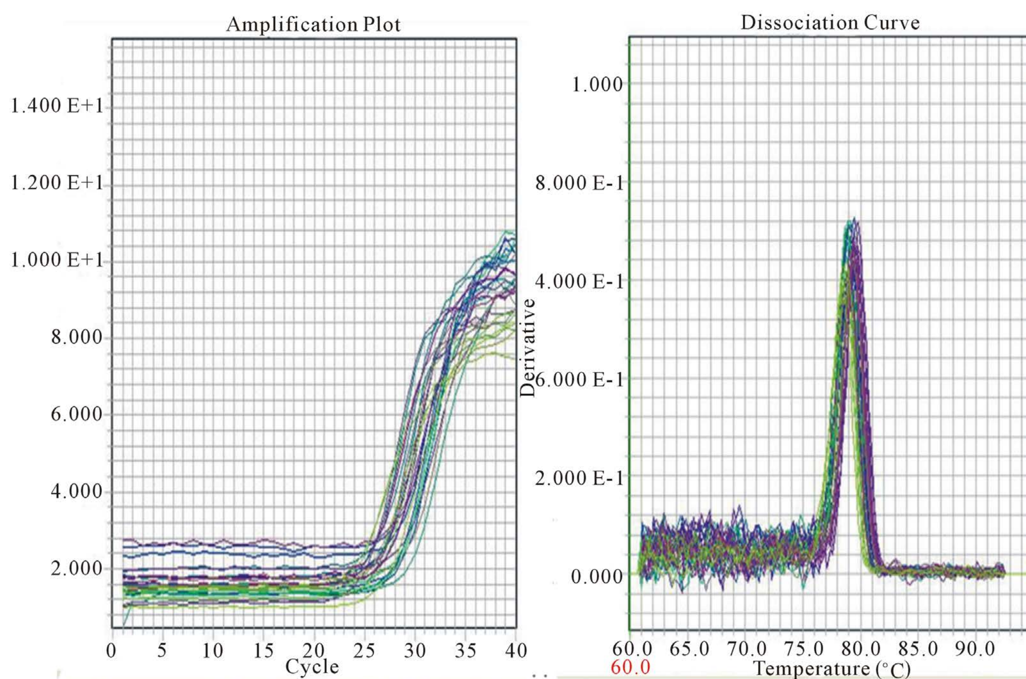

Figure 2. The amplification curve and the dissociation curve.

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Table 4. The expression of aurka gene in gastric cancer tissues in different clinicopathological characteristics

Characteristics	Number of patients	Expression of Aurka gene Mean ΔCT ± SD	T/F	P
Gender				
Male	41	7.13 ± 1.76	−0.09	0.93
Female	11	7.07 ± 1.20		
Age(year)				
~49	15	7.45 ± 2.20		
50 ~ 59	12	6.98 ± 1.66	0.28	0.84
60 ~ 69	15	6.90 ± 1.02		
70~	10	7.14 ± 1.67		
Stage				
I	15	6.21 ± 1.26		
II	10	7.12 ± 1.92	0.94	0.43
III	24	6.03 ± 2.16		
IV	3	6.84 ± 0.98		
Lymph node metastasis				
N0	20	6.31 ± 1.61		
N1	10	6.20 ± 2.27	0.21	0.90
N2	14	6.26 ± 2.05		
N3	8	6.84 ± 0.95		
Differentiation				
Moderate ~ well	16	5.69 ± 1.74	−1.88	0.07
Poor	36	6.68 ± 1.69		
Tumor's location				
Upper	13	5.99 ± 1.60		
Middle	14	6.50 ± 1.98	0.41	0.67
Lower	25	6.53 ± 1.79		
Depth of invasion				
T1 ~ T2	20	6.61 ± 1.31	0.21	0.81
T3 ~ T4	32	6.39 ± 2.01		
CEA				
(+)	6	7.52 ± 1.25	0.80	0.45
(−)	43	7.06 ± 1.69		
CA19-9				
(+)	8	8.04 ± 1.60	1.64	0.14
(−)	42	6.96 ± 1.62		

firmed it is an oncogene [7]. Nevertheless, no relationship was found between Aurka expression in gastric cancer tissues and pathological characteristics including tumor's location, depth of invasion, differentiation, lymph node metastasis and stage. HP infection is related to gastric canceration [19,20]. It is an important initiating factor of chronic atrophic gastritis, a precancerous disease of gastric cancer, and can induce the sequential event chronic atrophic gastritis developing to gastric mucosa intestinal metaplasia and to gastric cancer [21,22] speculated throught research result that HP infection could up regulate expression of Aurka, and participate in the early stage of gastric canceration. Our results, Aurka expressing notably higher in gastric cancer tissues of HP positive patients than HP negative ones, accorded with their conclusion. In addition, there was no relation between Aurka expression and gender, age, CEA and CA19-9 level in peripheral blood preoperation. CEA and

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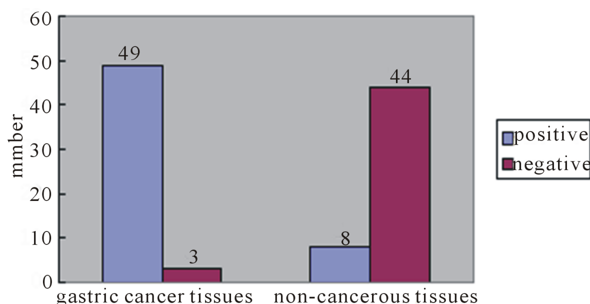
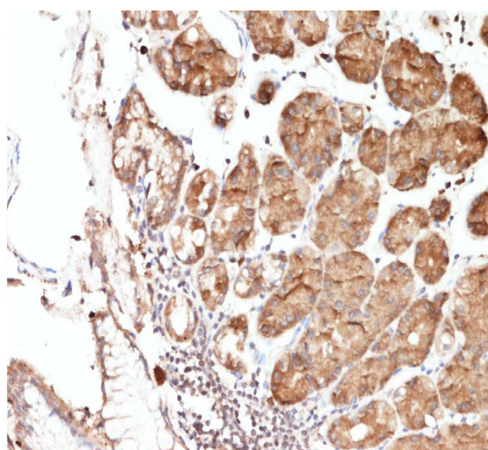
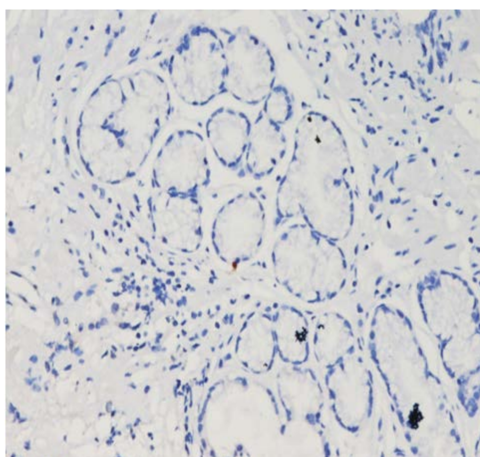


Figure 3. The expression of *aurka* protein in gastric cancer and corresponding non-cancerous gastric tissues



(a)



(b)

Figure 4. Aurka Protein Expression in Gastric Cancer Tissues (Positive) (a) and Corresponding Non-cancerous Gastric Tissues (Negative) (b) (both magnification × 200).

CA19-9 level in peripheral blood can be used to assess prognosis of gastric cancer patients [23,24] So it can be speculated that Aurka is unable to act as an indicator of gastric cancer prognosis .there is no unanimous conclusion that the mechanism of Aurka gene in gastric cancer, the development process, pending our further study. There has yet reports on Aurka gene and prognosis in patients with gastric cancer, therefore, to explore Aurka gene and prognosis of gastric cancer patients is worth further study. So follow-up to the patients in this study would be very important to make it clear.

5. CONCLUSION

Both Aurka gene and protein expressed significantly higher in gastric cancer tissues than paracancerous, which suggests that Aurka might play a significant role in the procession of the formation and development of gastric cancer as an oncogene. Its effect might be strengthened by HP infection. The expression level of Aurka gene had nothing to do with clinicopathological parameters including tumor's location, depth of invasion, differentiation, lymph node metastasis, stage; Also, it had nothing to do with clinicopathological parameters including gender, age and CEA, CA19-9 level in peripheral blood preoperation of gastric cancer patients, which suggests that although there has a closely relationship between Aurka and gastric cancer, it can't be as a prognostic indicator of relapse and speculate for gastric cancer.

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