Correlation between the Single Nucleotide Polymorphisms of CDH17gene and Gastric Carcinoma

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Abstract: Gastric cancer is one of the most frequently diagnosed malignancies in the world. The gene expression profile and molecular grouping of gastric cancer has been a challenging task due to its inherent complexity and variation among individuals.We sought to the correlation between the single nucleotide polymorphisms in extron 6 A58G site of CDH17 gene and gastric carcinoma.The method of the polymerase chain reaction - conformation polymorphism single-strand (PCR-SSCP) was used to detect genotype, combined with DNA sequencing was used to check the correctness of genotype.①The genotype frequencies of CDH17 in extron 6 A58G site: A/G type has 52 cases (76.47%), G/G type 11 patients (16.18%), A/A type 5 cases (7.35%). The allele frequencies of CDH17 in extron 6 A and G were 45.59%, 54.41% respectively.②The genotype frequencies and allele frequencies of CDH17 in extron 6 A58G site has no significant correlation with the gender, age, size, invasion depth, macroscopic appearance, histologic type and differentiation of gastric carcinoma ($P>0.05$). The genotype frequencies of CDH17 in extron 6 A58G site has correlation with lymph node metastasis and TNM grade of gastric carcinoma（$P<0.05$).③The allele frequencies of CDH17 in extron 6 A58G site has no significant correlation with the gender, age, size, invasion depth, macroscopic appearance, histologic type, differentiation, lymph node metastasis and TNM grade of gastric carcinoma($P>0.05$). The genotype frequencies and of CDH17 in extron 6 A58G site has correlation with lymph node metastasis and TNM grade in patients with gastric carcinoma.

[RenYin Chen, XiaoBo Liu, Juan Chen, JuanJuan Cao, JianPing Yang, Hui Meng, GuoQiang Zhao, YuFeng Zhang. Correlation between the Single Nucleotide Polymorphisms of CDH17gene and Gastric Carcinoma. *Life Sci J* 2012; 9(4):299-303]. (ISSN: 1097-8135). http://www.lifesciencesite.com. 44

Keywords: Gastric Carcinoma; the CDH17 Gene; Single Nucleotide Polymorphism

1. Introduction

Gastric cancer (GC) is a world health burden, ranging as the second cause of cancer death worldwide[1]. Etiologically, GC arises not only from the combined effects of environmental factors and susceptible genetic variants but also from the accumulation of genetic and epigenetic alterations[2].In the world's wide gastric carcinoma's death rate only below lung cancer, colorectal and breast cancer. It's five years survival rate is below 20%[3]. Single nucleotide polymorphisms are the third generation of genetic markers, are the most important genetic change in humans. SNPs have characters of large quantity, stabilization and feasibility for detecting[4]. Many SNP sites have correlation ship with gastric carcinoma. CDH17 gene is a special member of cadherin superfamily. It is a gastric cancer related gene, which has its own characteristics in mediated cell adhesion[5]. We explored the correlation between the single nucleotide polymorphisms in extron 6 A58G site of CDH17 gene and gastric carcinoma.

2. Material and Methods

Clinical materials

The peripheral venous blood samples were collected from 68 unrelated patients with gastricl carcinoma in Henan province, China. The time of collecting samples were from December 2009 to April 2010. All patients with gastric carcinoma had treatments in the first affiliated hospital of Zhengzhou University. 1 mL Blood samples were collected from patients before surgery.

Method to identifying SNP genotypes

DNA extraction kits of spin column type was used to extract DNA. The method of the polymerase chain reaction - conformation polymorphism single-strand (PCR-SSCP) was used to detect genotype, combined with DNA sequencing was used to check the correctness of genotype.

Method of statistical analysis

SPSS17.0 statistical software package was used. About unordered categorical datas, analyse them by $\chi 2$ test, Odds ratio(OR) and 95% confidence interval(95%CI) were used to express risk degree(OR

and 95%CI were computed by Logistic regression model). About ordered categorical datas, analyse them by rank sum test, the mean ranks were used to express risk degree. Using two-sides probability test when there were two kinds of side tests. Significant level was 0.05. Method of Bonferroni was used to mutiple comparision.

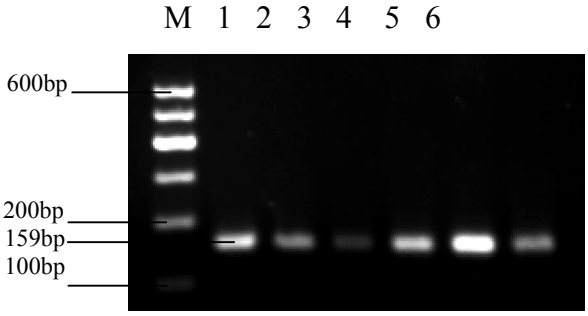
3. Results

The result of genomic DNA extracted

Genomic DNA extracted by uv spectrophotometry identification evaluation, the A 0D260 of sample DNA is greater than 1.1 and A OD 260/ 280nm all in between 1.7-1.9.

The result of PCR

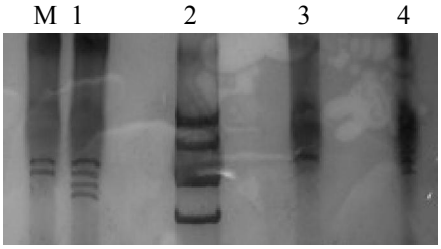
PCR products by 1.5% agarose gel electrophoresis, automatically gel imaging system observations. With DNA marker in the 159bp can saw a clear strip (Fig1).



M：DNA Marker（600bp 至 100bp）；
1-6 samples

The result of polyacrylamide gel electrophoresis

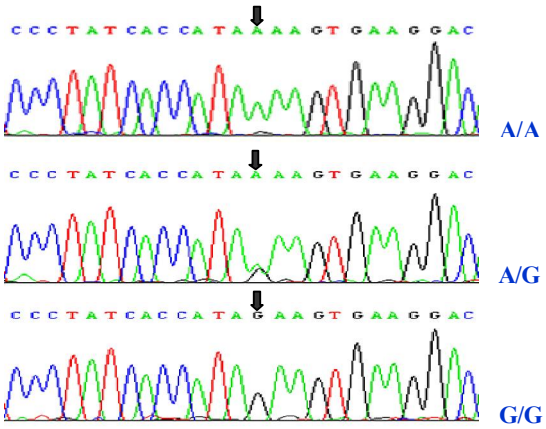
PCR products after the thermal deformation by polyacrylamide gel electrophoresis, then silver stain the gel observaed .According to results observed to judge genotype (Fig 2)



M：DNA Marker（200bp 至 600bp）；1、2、 3、 4 samples

The result of DNA sequencing

Select 10 cases have representative samples (including the belt type unexplained sample) for DNA sequencing. According to the result observed to judge genotype(Fig 3). Arrow signed for this single nucleotide polymorphisms (SNPs).



The result of statistical analysis

The correlation between the single nucleotide polymorphisms in extron 6 A58G site of CDH17 gene and gastric carcinoma.

The genotype frequencies of CDH17 extron 6 A58G site: A/G type has 52 cases (76.47%), G/G type 11 patients (16.18%), A/A type five cases (7.35%). The allele frequencies of CDH17 exon 6 A and G were 45.59%, 54.41% respectively. he genotype frequencies and allele frequencies of CDH17 extron 6 A58G site has no significant correlation with the gender, age, size, invasion depth, macroscopic appearance, histologic type and differentiation of gastric carcinoma ($P>0.05$). The genotype frequencies of CDH17 extron 6 A58G site has correlation with lymph node metastasis and TNM grade of gastric carcinoma（$P<0.05$）(Table 1).

Table 1. The correlation between CDH17gene in extron 6A58Gsite and clinicopathologic parameters

Clinicopathologic parameters	*n*	Genotype			χ^2	*P*
		G/A	G/G	A/A		
Gender						
male	41	31	7		0.062	0.970
female	27	21	4			
Age (year)						
＜50	11	8	2	1	0.111	0.946
≥50	57	44	9	4		
Tumor size(cm)						
＜5	23	18	4	1	0.473	0.789
≥5	45	34	7	4		
Macroscopic appearance	12	9	2	1		
Protrude typ					1.214	0.786
Ulcera type	42	32	7	3		
Infiltrating type	14	11	2	1		
Histologic type						
adenocarcinoma	41	32	5	4		
adenocarcinoma and mucinous carcinoma	16	12	3	1	1.277	0.865

mucinous carcinoma	11	8	3	0		
Invasion depth						
≤muscular layer	26	21	2	3	0.207	0.902
＞muscular layer	42	31	9	2		
Differentiation degree						
well differentiated	7	4	2	1		
moderately differentiated	9	6	2	1	1.137	0.566
poorly differentiated	52	42	7	3		
lymph node metastasis						
no	31	21	9	1	1.957	0.036
yes	37	31	2	4		
TNM stage						
Ⅰstage	7	5	2	0		
Ⅱstage	28	23	4	1	4.053	0.032
Ⅲstage	21	16	4	1		
Ⅳstage	12	8	1	3		

4. Discussions

CDH17 namely cadherin 17, also known as LI - cadherin or liver - intestine cadherin located in 8q22.1[6]. Its a special member of cadherin superfamily, which has its own characteristics in mediated cell adhesion[7].Reserch has confirmed that LI - calcium sticky protein not through serial protein to connect with cytoskeleton actin , likely to be directly connected with the cytoskeleton .It has a important synergy effect on the adhesive attraction of the Classic calcium sticky protein. In normal tissue it take part in the integrity maintenance of the liver and intestinal epithelium cells[8] . Normally LI - calcium sticky protein expressed in liver cell and the intestinal mucosa ,in gastric mucosal surface is not expressed. Along with the progress of gastric cancer, LI - calcium sticky protein expression shows ascendant trend, in advanced intestinal-type gastric carcinoma LI – calcium expressed more than the early. LI - calcium sticky protein expression is more, the tumor is deeper infiltrated, the possibilities of lymph node metastasis is higher[8-13].

Nowtimes the study about the correlation between the SNPs of CDH17 gene and the carcinoma is not so many. Liu QS et al [14]results showed that a well-differentiated gastric cancer cell line had higher CDH17 expression. Down-regulation of CDH17 inhibited proliferation, adherence, and invasion of the poorly differentiated BGC823 gastric cancer cells in vitro, and induced cell cycle arrest.they confirmed that CDH17 silencing could obviously slow the growth of gastric cancer derived from BGC823 cells. Taken together,they have demonstrated that CDH17 maybe a positive regulator for proliferative, adhesive, and invasive behaviors of gastric cancer. Wang[15] et al research 164 patients with HCC patients 99 cases with cirrhosis of the liver and 293 example of healthy people found: LI - calcium glue CDH17 (651C > T protein. 35A > G) IVS6 + the T - G single figure is a genetic sensitive factor of hepatocellular carcinoma in China crowds. Inoue M[16]et al study showed the grade of BilIN independently correlates with LI-cadherin expression in biliary intraepithelial lesions of ICC without hepatolithiasis.Lee NP[17], et al have reported aberrant expression of CDH17 in major gastrointestinal malignancies including hepatocellular carcinoma (HCC), stomach and colorectal cancers, and its clinical association with tumor metastasis and advanced tumor stages. Furthermore, alternative splice isoforms and genetic polymorphisms of CDH17 gene have been identified in HCC and linked to an increased risk of HCC. CDH17 is an attractive target for HCC therapy. Targeting CDH17 in HCC can inhibit tumor growth and inactivate Wnt signaling pathway in concomitance with activation of tumor suppressor genes. Weimann A et al[18] using immunohistochemistry detected LI-cadherin in esophageal adenocarcinoma (n = 16),.in adenocarcinoma, the expression of LI-cadherin was significantly weaker or absent.

Lee HJ,et al [19]reported expression of CDH17 was up-regulated in gastric cancer tissues. using immunohistochemistry showed that CDH17 was an independent prognostic factor in patients with stage I or node-negative disease. So CDH17 is a promising prognostic marker for early stage gastric cancer. Su MCet al[20] using immunohistochemistry showed that CDH17 Fewer than 1% of carcinomas outside the digestive system were positive for cadherin-17.the results show that cadherin-17 is a useful immunohistochemical marker for diagnosis of adenocarcinomas of the digestive system.Yasui W et al[21] expression of CDH17 were associated with an intestinal phenotype of gastric cancer. Ge Jet al[22] reported the expression of CDH17 is associated with the intestinal-type gastric carcinoma. Positive expression of CDH17 was significantly associated with the depth of gastric wall invasion, lymph node metastasis and stages of gastric carcinoma. The expression of CDH17 was significantly lower in diffuse-type carcinoma than intestinal- or mixed-type carcinoma. The patients with CDH17 expression associated with poor prognosis of gastric carcinoma,. The survival rate of patients with CDH17expression was the lowest. Dong WG et al[23] investigated expressions of Li-cadherin in gastric cancer by immunohistochemistry and semiquantitative polymerase chain reaction (PCR), and correlated this with clinicopathologic parameters in 91 cases of gastric cancer. the expression level of Li-cadherin mRNA was correlated to differentiation and lymph node metastasis, and the expression level of Galectin-3 was related to TNM staging, differentiation and lymph node metastasis. On Spearman correlation analysis, a definitive negative correlation was found

between the expression levels of Li-cadherin in gastric cancerous tissues.

Sakamoto N[24] analyzed gene expression profiles of HT-29 cells treated with EGFR ligands and identified 6 genes up-regulated by epidermal growth factor (EGF) and Transforming growth factor (TGF)-α treatment. Significant correlation was found between LI-cadherin expression and advanced T grade and N grade. Both EGFR and LI-cadherin expression was more frequently found in GC cases with an intestinal mucin phenotype than in cases with a gastric mucin phenotype. These results indicate that, in addition to the known intestinal transcription factor caudal type homeobox 2 (CDX2), EGFR activation induces LI-cadherin expression and participates in intestinal differentiation of GC.But we only finded G/G, G/A, A/A subtypes in gastrical carcinoma.

CONCLUSION:

The results suggest that the expression of CDH17 or CDX2 may be an important feature of gastric carcinoma. A combined detection of CDH17/CDX2 co-expression may benefit us in predicting the prognosis of gastric carcinoma.

For LI - calcium sticky protein gene explicit son 6A58G sites polymorphism and gastric carcinoma research did not see the relevance of reports. This experiment chose 68 cases of gastric cancer patients in Henan province as the research object, the results show: the genotype frequencies of CDH17 extron 6 A58G site has no significant correlation with the gender, age, size, invasion depth, macroscopic appearance, histologic type and differentiation of gastric carcinoma . the genotype frequencies and of CDH17 extron 6 A58G site has correlation with lymph node metastasis and TNM grade in patients with gastric carcinoma. This research conclution is agreed the findings of Ko[25] et al that LI - calcium sticky protein expression and gastric cancer pathological staging was associated with the lymph node metastasis. Gastric carcinoma lymph node metastasis and stomach TNM staging is closely related to the prognosis of patients with stomach cancer, and therefore may consider LI - calcium sticky protein as gastric lymph node potential transfer risk and the tumor 5-year survival rate judgment with gastric carcinoma patients.

Tan IB[26] et al have reported that intrinsic subtypes of GC, based on distinct patterns of expression, are associated with patient survival and response to chemotherapy. classification of GC based on intrinsic subtypes might be used to determine prognosis and customize therapy. Zhang J et al [27] reported Liver-intestine cadherin (CDH17) is a novel member of the cadherin superfamily implicated in gastric cancer progression. RNA interference mediated by recombinant lentivirus vectors expressing artificial CDH17 miRNA was applied to induce a long-lasting down-regulation of CDH17 gene expression in BGC823 cells. The expression levels of CDH17, tumor cell motility, migration potential, and proliferation were measured by flow cytometry, real-time RT-PCR, Western blot analysis, immunofluorescence staining, wound healing assay, and MTT assay, respectively. Results show that four recombinant plasmid expression vectors encoding pre-miRNA against CDH17, pcDNA-CDH17-miR-SR1, -SR2, -SR3, and -SR4 were constructed correctly and down-regulated the CDH17 mRNA levels by 5.5, 57, 91, and 98%, respectively, in BGC823 cells which had an overexpression of CDH17. They packaged the recombinant lentiviral vector for CDH17 RNA interference with pcDNA-CDH17-miR-SR4 which had the highest interfering efficiency and succeeded in construction of the stable transfectants. Of note, more than 90% knockdown of CDH17 expression in BGC823 cells was obtained by miRNA technique. The CDH17-miRNA-transfected cells showed significant decrease in cell proliferation, cell motility, and migration in comparison with the control cells. Thus, They proposed that CDH17 may be an oncogene up-regulating invasive features of gastric cancer cells and could be a hopeful target for the control of gastric cancer progression.

To sum up, the genotype frequencies and of CDH17 extron 6 A58G site has correlation with lymph node metastasis and TNM grade in patients with gastric carcinoma. This will help to evaluate gastric carcinoma patients lymph node metastasis and 5-year survival rate.

Acknowledgments

We thank Yuanting gu for their wisdom and guidance in the retrieval and evaluation of these samples, and Shuijun Zhang , Shaomin Zhang for assistance with acquisition of historical reference. This paper is written in tribute to the memory of Prof Sugimura and with respect to other former faculty members and staff in the Department of Pathology at the First Affiliated Hospital of Zhengzhou University for the care and preservation of records and tissue samples from the preintensivist era. We particularly thank Dondling Gao who worked on the technical aspects of tissue retrieval and preparation .

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Grant Support

This work was supported in part by 2010A310011 Henan provincial department of education on natural science research projects.

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8/29/2012