SIGNIFICANCE OF THE *K-RAS* GENE CODON 12 POINT MUTATION IN STOMACH CANCER IN SOUTHERN INDIA

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ABSTRACT: The frequency and clinicopathologic significance of the K-ras gene point mutation in stomach cancer remain to be defined. We investigated the frequency of K-ras codon 12 point mutations in stomach cancer using a polymerase chain reaction (PCR)-based method in 94 samples and 100 age and sex matched controls. The overall frequency of K-ras codon 12 point mutations in stomach cancer was 3.19% (3/94). DNA sequencing of three cases with K-ras codon 12 point mutations identified a single-base substitution of GGT to GTT (glycine to valine) .Two of them were in heterozygous condition and one was in homozygous condition. Conclusions: K-ras codon 12 point mutations are uncommon in stomach cancer (3.19%) in Southern India.

Key words: K-ras, codon12,gastric cancer, significance

INTRODUCTION: It is now well known that ras oncogenes play an important role in the pathogenesis of various types of cancer. Point mutations at codons 12, 13, and 61 of ras genes result in equilibrium shift of Ras proteins towards the activated state, which constitutively activates the mitogenic signal transduction pathway .Frequency of mutated Ras genes varies among the different tumor types. Point mutations of the K-ras gene are found predominantly in adenocarcinomas. The highest incidence is found in adenocarcinoma of the pancreas, in which about 90% of the tumors harbor a mutated K-ras gene. According to the report by Almoguera et al 1988, 21of 22 carcinomas of the exocrine pancreas contained K -ras genes with mutation at codon 12. Almost all K-ras gene mutations in pancreatic cancer occur at codon 12.In colorectal carcinoma, about 50% of the tumors show K-ras mutations and more than 70% of the mutations are located at codon 12. (Vogelstein B 1988, Bos JL 1987). .

Molecular events in the pathogenesis of stomach cancer are largely unknown. Several studies reported low incidence of Ras gene mutation in gastric cancer, although most studies analyzed only a small number of cases. The frequency of K-ras gene mutation in stomach cancer remains to be defined.

To ascertain the frequency of K-ras mutation in stomach cancer, one must analyze a large number of samples using a sensitive detection method. In the current report, we used a polymerase chain reaction (PCR) method" to detect K-ras codon 12 mutation in a series of 94 patients. The findings were correlated with various clinicopathologic characteristics including tumor site,tumor stage and tumor type.

MATERIALS AND METHODS:

Patient Selection

Between January 2003 and November 2003, a series of 94 patients who were diagnosed with stomach cancer at the Osmania General Hospital, Hyderabad were enrolled in a prospective study investigating prognostic factors in stomach cancer. The samples were frozen rapidly in polypropylene screwcap tubes and stored at -80^{0}C .Various clinical characteristics of the patients, such as age, sex, duration of symptom, history of upper gastrointestinal bleeding, history of weight loss were noted.Weight loss was determined to be present if the patients lost over 10% of their body weight during a 6-month period before the diagnosis. Various pathologic characteristics of the tumors, such as tumor size, location in the stomach,stage, and histologic differentiation, were noted.The location of the tumors was determined after a review of the surgical and gross pathologic findings. The tumors located predominantly in the gastroesophageal junction and cardia were determined to be in the upper third of the stomach. The tumors located in the midbody and pylorus were determined to be in the middle third and lower third of the stomach, respectively. The tumors were staged according to Lauren's classification.

DNA Preparation

To the homogensed tissue (2-2.5 mg) ,tissue lysis buffer ((Tris hydrogen chloride 10 mmol,magnesium chloride 0.15 mmol, potassium chloride 50 mmol, gelatin 0.1 mg/ml, pH 8.3), 10% SDS and Proteinase K (20mg/ml) were added and incubated at 37^0 C until the pieces disappeared and a uniform solution was obtained .To this solution equal amount of phenol was added and mixed gently followed by centrifugation . Supernatant was retained and DNA was precipitated using ethanol followed by 70% ethanol wash ,air drying and dissolving in nuclease free water.

Synthesis of ***Primers***

Oligonucleotide primers near codon 12 of the K-ras gene were synthesized by Bioserve India Pvt Ltd (India). The sequences of primers and the PCR conditions are listed in Table 1.

Table I : PCR Conditions

K-ras For	5-ATAGTTTGAAGTGCCTGTTTGG-3	94 -7 min ,30 cycles of 95 ◦*C for 5 s, 58* ◦*C for 20 s, 72* ◦*C for 60 s, and 72* ◦*C for 6 min.*
Rev	5-GAGTGGTCATTTTTAATGTTTG-3	

Polymerase Chain Reaction

PCR reactions were set up with the above primers and specified conditions as given in Table 1 in a thermocycler (Eppendorf master cycler gradient) . The amplified products were screened for the presence of mutations by Restriction fragment polymorphic method (RFLP).

Restriction Fragment Length Polymorphism Analysis

The PCR product was digested by restriction enzyme BstXI (Fermentas,# FD1024,1FDU/ul). The restriction fragment length polymorphism (RFLP) analysis was done as follows; Enzyme 2ul (enz:buff-1:5);PCR product- 2ul. The reaction time was 2 hours at 37^0 C. After digestion products were visualized on a 2% agarose gel .The products identified as having mutations were sequenced.

RESULTS:

We found 3 stomach cancers harboring K-ras codon 12 point mutation among 94 samples (3.19%, Fig. 2). At codon 12, a G>T transition was found which changed the amino acid from glycine to valine (GGT /GTT). (Figure-**1)**

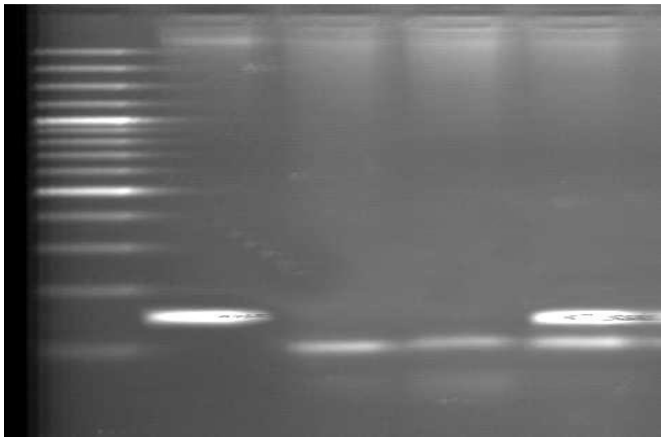

Figure 1: Agarose gel photograph of RFLP analysis of K-Ras amplicon. Out of three patients identified with mutations , 1 was a heterozygote (Patient 23)(GT; 106bp+134bp) lane 5 ; 2 were with recessive genotype (TT;106bp) (patients 55 and 69) in lanes 3 and 4 ; (Lane 1: 100 bp marker ,lane 2 : Wild type(GG):134bp

Relative risk estimate analysis

The relative risk estimate of the genotypes found in our study showed no significance between cancer patients and controls. (Table 2)

Table 2: Relative risk estimate of the genotypes in cancer and control groups

Genotype	Cancer	Control	chi- square value
GT	3	0	2.09
GG	91	100	----

DISCUSSION:

The purpose of our study was to determine the frequency of K-ras codon 12 point mutation in stomach cancer in a South Indian population and to compare these results with those that have been obtained in other populations. We used a sensitive PCR-based method that could detect less than 2% of mutant DNA mixed in wild-type DNA Our finding is in agreement with other data that showed relativelylow frequency of ras genes mutation in stomach cancer when compared other gastrointestinal tumors, such as pancreas, biliary, and colorectal cancers. The data from 10 previous studies on *ras* gene mutation in stomach cancer are summarized in Table 3.

Table 3. Data From 11 Studies on *ras* Gene Mutation in Stomach Cancer

Gene Incidence	(%)	**Method**	**Reference no.**
K-ras	1/37(2.7)	PCR, ONH	Koshiba M et al ,1993
14			
N-ras	0/37(2.0)		
H-ras	0/37 (0)		
K-ras	3/32 (9.4)	PCR,DGGE	Ranzani GN et al ,1993
K-ras	3/35 (8.6)	PCR, ONH	Kihana T et al,1991
K-ras	4/33 (12.1)	PCR, ONH	Miki H et al,1991
H-ras codon 12	6/17 (35.3)	PCR, RFLP	Deng G et al,1991
K-ras	0/11 (0)	PCR,ONH	Victor T et al, 1990
N-ras	0/11 *(0)*		
H-ras	0/11 (0)		
K-ras	1/28 (3.6)	PCR, ONH	Nanus DM et al,1990
N-ras	1/28 (3.6)		
H-ras	0/28 (0)		
K-ras	0/27 (0)	PCR,RFLP	Jiang W et al, 1989
H-ras	0/27 (0)		
H-ras	1/8 (12.5)	PCR,ONH	Koh EH et al,1992
H-ras,codon 12	0/7 (0)	TA	Levy S et al,1991

ONH: oligonucleotide hybridization; **DGGE**: denaturing gradient gel electrophoresis; **TA:** transfection assay with NIH/**3T3** cells; **PCR:** polymerase chain reaction; **RFLP**: restriction fragment length polymorphism analysis.

Overall frequency of K-ras codon 12 mutation in the previous studies and our study are not significantly different. This suggests that geographical and ethnic variation may not affect the frequency of K-ras mutations in pathogenesis of gastric cancer. An explanation for this similarity in incidence rates may be due to similar pathogenic mechanisms of stomach cancer observed.

Conclusion: In conclusion, the overall frequency of K-ras codon 12 mutation in stomach cancer in South Indian population is 3.19%. Further studies concerning oncogenes, including *ras* genes, are warranted to elucidate molecular pathogenic mechanisms of stomach cancer.

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REFERENCES

Almoguera C, Shibata D, Forrester *K,* Martin J, Arnheim *N ,* Perwho M. Most human carcinomas of the exocrine pancreas contain mutant *c-K-ras* genes. *Cell* 1988;53:549-54.

Bos JL, Fearon ER, Hamilton SR, Verlaan-de Vries M, Van Boom JH, Van der Eb AJ, et al. Prevalence of *ras* gene mutations in human colorectal cancers. *Nature* 1987;327:293-7.

Deng G, Liu X, Wang J. Correlation of mutations of oncogene CHa-ras at codon 12 with metastasis and survival of gastric cancer patients. Oncogene Res 1991;6:33-8.

Fujita K, Ohuchi N, Yao T, Okumura M, Fukushima Y, Kanakura *Y ,* Frequent overexpression, but not activation by point mutation,of ras genes in primary human gastric cancers. Gastroenferology 1987;93:1339-45.

Jiang W, Kahn SM, Guillem JG, Lu S-H, Weinstein IB. Rapid detection of *ras* oncogenes in human tumors: application to colon, esophageal, and gastric cancer. *Oncogene* 1989;4:923-8.

Koh EH, Chung HC, Lee KB, Han EK, Oh SH, Min JS, et al. Point mutation at codon 12 of the c-Ha-ras gene in human gastric cancer. JKoreanMedScience 1992;7:110-5.

Koshiba M, Ogawa 0, Habuchi T, Hamazaki S, Shimada T, Takahashi R, et al. Infrequent ras mutation in human stomach cancers. ***Jpn*** J Cancer Res 1993; 84:163-7.

Kihana T, Tsuda H, Hirota T, Shimosato Y, Sakamoto H, Terada M, et al. Point mutation of c-Ki-ras oncogene in gastric adenoma and adenocarcinoma with tubular differentiation. ***Jpn*** *J* Cancer Res 1991;82:308-14.

Nanus DM, Kelsen DP, Mentle IR, Altorki N, Albino AP. Infrequent point mutations of ras oncogenes in gastric cancers. Gastroenterology 1990; 98:955-60.

Ranzani GN, Renault B, Pellegata NS, Fattorini P, Magni E, Bacci F, et al. Loss of heterozygosity and K-ras gene mutations in gastric cancer. Hum Genet 1993;92:244-9.

Victor T, Du Toit R, Jordaan AM, Bester A], van Helden PD. No evidence for point mutations in codons 12, 13, and 61 of the ras gene in a high-incidence area for esophageal and gastric cancers. Cancer Res 1990;50:49 11-4.

Vogelstein B, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, et al. Genetic alterations during colorectal-tumor development. N *Engl* ***IMed*** 1988;319:525-32.

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