Glycine to Aspartic Acid Mutations at Codon 13 of the c-Ki-ras Gene in Human Gastrointestinal Cancers

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ABSTRACT

Point mutations of c-ras genes were analyzed in human gastrointestinal cancers. DNA obtained from the tissues was amplified by polymerase chain reaction and then analyzed by dot blot hybridization assay with oligonucleotide probes to detect mutations at codons 12, 13, and 61 of c-Ki-ras, c-Ha-ras, and c-N-ras. In two of 25 cases of stomach cancer point mutations at codon 13 of c-Ki-ras were found. In colorectal cancer, eight of 30 cases showed mutations: four cases of codon 12 and one case at codon 13 of c-Ki-ras and two cases at codon 61 and one case at codon 13 of c-N-ras. These results may indicate involvement of a wide variety of c-ras gene point mutations, in addition to those at codon 12 of c-Ki-ras, in oncogenesis of human gastrointestinal cancers. In all three mutations of c-Ki-ras at codon 13 which had been seldom found in human cancers, glycine to aspartic acid mutations due to identical G to A transition at the second nucleotide were observed.

INTRODUCTION

Analysis of cellular DNA of human cancer cells in transformation assays identified activated c-ras genes with point mutations (1). These point mutations were so far observed only at codons 12, 13, and 61 of the c-ras gene family although mutations in other codons were shown to lead to activation of genes in in vitro experimental systems. The more recently devised PCR and hybridization assays with oligonucleotide probes allowed analysis of precise profiles of point mutations by examining a wide variety of human cancer tissues as well as cell lines (2).

Analyses of an extensive number of human cancer tissues, particularly those of digestive organs such as colorectal cancer (3, 4) and pancreatic cancer (5), identified frequent mutations of c-Ki-ras at codon 12, confirming results of the initial analysis of a limited number of samples utilizing transformation assays. Since the activation of c-Ki-ras by mutations at codon 12 was also found in other types of human cancer such as lung cancer (6), this particular codon seemed to be a preferential and probably critical target of point mutations in certain epithelial cell cancers.

We report here our analysis of 25 cases of human stomach cancer and 30 cases of human colorectal cancer in terms of point mutations at codons 12, 13, and 61 of c-Ki-ras, c-Ha-ras, and c-N-ras genes. We found a variety of point mutations in gastrointestinal cancers including those at codon 13 of c-Ki-ras which had been previously seldom found in human cancers.

RESULTS

Analysis of Point Mutations in c-ras Genes in Human Gastrointestinal Cancers. Genomic DNA obtained from either frozen tissues or 10% formalin-fixed and paraffin-embedded tissues were amplified by PCR. Point mutations of the c-ras family were examined by dot blot hybridization assays with oligonucleotide probes. In two of 25 cases of stomach cancer, point mutations of c-Ki-ras at codon 13 were found as shown in Fig. 1 and summarized in Table 1.

In colorectal cancer, eight of 30 cases showed mutations; four cases at codon 12 and one case codon 13 of c-Ki-ras and two cases at codon 61 and one case at codon 13 of c-N-ras. Examples of dot hybridization assays are also shown in Fig. 2 and results are summarized in Table 2. In both types of cancer, normal
counterpart epithelial tissues were obtained from most patients whose cancer tissues showed point mutations. No mutation was detected in the counterpart tissues. In both types of cancer, no mutation was detected at the second nucleotide (data not shown). This should confirm results obtained by dot hybridization analyses. In our analysis of human gastrointestinal cancers, point mutations at codons 12, 13, or 61 of the c-ras family were found.

**DISCUSSION**

In our analysis of human gastrointestinal cancers, point mutations at codons 12, 13, or 61 of the c-ras family were found.
in two of 25 cases of stomach cancer and eight of 30 cases of colorectal cancer. According to our analysis of sizable numbers of stomach cancer, the frequency of the c-ras gene family at these examined sites seems to be relatively low. This may well agree with the report by Sakamoto et al. (15) who found no case with activated c-ras genes in 26 cases of stomach cancer in colorectal cancer. According to our analysis of sizable numbers contrast to the previous report by Smit et al. (16) who found G Japanese. In this regard, it should be described that in our essentially compatible with those results by Bos et al. (3) who agree with the report by Sakamoto et al. (15) who found no these examined sites seems to be relatively low. This may well of stomach cancer, the frequency of the c-ras gene family at in two of 25 cases of stomach cancer and eight of 30 cases of gastrointestinal cancers. In evaluation of mutations of the c-ras gene family in This seems to be particularly the case in stomach cancer. Reasons to adequately explain the differences between previous reports and ours are unknown as yet. Genetic differences which might influence patterns of mutations might be one factor taken into consideration since all our samples were obtained from the Japanese. In this regard, it should be described that in our analysis of 38 Japanese cases of pancreatic cancer, G A transitions at codon 12 of c-Ki-ras were frequently observed in contrast to the previous report by Smit et al. (16) who found G to T transversions were dominant.

Since the initial analyses of human cancers in in vitro and in vivo transformation assays, point mutations have been dominant at codons 12 and 61 in the entire c-ras gene family. In fact, point mutations at codon 13 of c-Ki-ras and c-Ha-ras have seldom been found in previous studies (17–20), while mutations at codon 13 of c-N-ras have been detected with relatively higher frequency in myelogenous malignancies (21–23). Although dominance of mutations at codons 12 and 61 might be still the case in general, our results have shown that mutations of c-Ki-ras at codon 13 may not be rare, at least in gastrointestinal cancers. In evaluation of mutations of the c-ras gene family in human cancer, mutations at codon 13, in addition to those at codons 12 and 61, must be carefully examined since they might be more frequent than previously observed in various human cancer tissues.

Another point of considerable interest is the fact that all point mutations we found at codon 13 of c-Ki-ras gene were G to T transitions of the second nucleotide both in stomach and colorectal cancer. Interestingly, previous reports on c-Ki-ras gene mutations at codon 13 also showed identical changes (17–19). This may strongly indicate that G to T transition of the second nucleotide may take place with high frequency at codon 13 of c-Ki-ras. Alternatively, these results may indicate that a glycine to aspartic acid change is critical for c-Ki-ras genes to become activated as a consequence of point mutations at codon 13. It is therefore essential to analyze functional aspects of c-Ki-ras with a series of point mutations at codon 13. The study is being done in our laboratory.

In our present study, we failed to ascertain the particular association of c-ras mutations with histological types of gastrointestinal cancers. It will be necessary, however, to examine larger numbers of cases in order to conclude whether presence or type of c-ras mutations is associated with any biological characteristics of these cancers such as prognosis and histology.

REFERENCES

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