Abstract

Topic: "Molecular Analysis of p53 tumor suppressor gene in Human Gastric Cancer"

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Gastric cancer is a common disease worldwide and also one of the leading causes of cancer death (5^{th} in male and 6^{th} in female) in India. The estimated number of new cases each year is expected to rise from 10 million in 2000 to 15 million by 2020. Stomach cancer is the second most frequent cancer in the world (Parkin *et al.,* 2005). The molecular biology of gastric cancer has been widely studied in the developed world, only few scattered reports from the developing world and there is no published data reported from India. The molecular events leading to the developments of gastric cancer are largely unknown, but there is now enough evidence to suggest that the functional inactivation of p53 gene through allelic loss and point mutation plays an important part. Previous studies suggest that the frequency of p53 alteration in gastric cancer varies from 5% to 70%. However, the clinical significance of p53 is still a subject of debate. Thus the present study was aimed to analyze the alterations in p53 gene among Indian gastric cancer patients and to correlate them with the various clinicopathological parameters. The work was carried out with the following objectives-

- Collection of tissues samples from gastric cancer patients
- Clinico-pathological studies (Histopathology and immunohistochemistry).
- Determination of mutational status of p53 gene in these samples using PCR-SSCP and DNA sequencing analysis.
- Correlation of p53 alteration with the specific etiological factors along with different stages of cancer.

Between April 2002 to April 2006, 128 consecutive patients tissue with suspected gastric cancer provided by general surgeons were enrolled for study. A detailed histopathological examination was performed to determine the depth of invasion on the gastric wall and the extent of metastases within regional lymph nodes. Among 128 cases, 25 were found to be histopathologically negative for gastric cancer and thus were excluded from the study. The remaining 103 cases were from West-Bengal (n = 60), Jammu & Kashmir (n = 30) and New Delhi (n = 13). Patients includes male (n = 78) and female (n = 25) with mean age of 56 years (range 25-71 years). The p53 alterations were studied by both immunohistochemical method as well as PCR-SSCP analysis, followed by nucleotide sequencing. We only studied four (exon 5, 6, 7, and 8) of the 11 p53 exons as it is reported that more than 95% of alterations occur at these four exons only.

Clinicopathological data revels that occurrence of gastric cancer is thrice as common in men as in women and peak incidence level found at 60 years of age where young people (<40 year) have low frequency. A significant correlation of young gastric cancer patients (< 40 yr of age; n=13) were found with stage of cancer (P= 0.01) and diffused type cellular differentiation (P = 0.048). No major differences in risk were seen according to location of stomach. Among 103 cases, p53 overexpression and mutation were detected in 37 (35.92%) and 19 (18.44%) cases, respectively. Most of the p53 mutation were missense (16/19) and found at exon 5 (31.54%), followed by exon 6 (26.31%), exon 7 (21.04%) and exon 8 (21.04%). A significant correlation of p53 overexpression was found with mutation (P = 0.000), gender (P = 0.004) and histological subtype (P =

0.001). Concordance between p53 alteration (as detected by SSCP) and overexpression (as detected by IHC) was found in 75% cases. We found that IHC-positive/SSCP-negative cases accounted for 21% of cases and IHC-negative/SSCP-positive cases accounted for remaining 4% cases. While examining the mutation spectrum of adenocarcinoma of the stomach we found that there is a high prevalence of transition mutations 73.68 % (14 of the 19) than transversion 35.71 % (5 out of 19). 57.14 % (8/14) were either from G→A or from A→G transitions and 42.85% (6/14) were either C→T or T→C transition. G→A or A→G transition of mutation is commonly believed to be the effect of an external carcinogen. There were two nonsense mutations detected at codon 196 and 213 (Arg to Stop) of exon 6 and one silent mutation at codon 293 (Gly – Gly) of exon 8 of p53 gene. 6 out of 14 transitions were at CpG dinucleotides. All the transversion mutations were found in the antrum region of stomach whereas the transitions were found distributed in all the four sites of stomach.

Our findings add to the evidence that diet (salted tea, dry fish, poppy seed, deep fried rice/ meat and pickle) plays a major role in stomach cancer risk and suggest the need for further evaluation of risks associated with carbohydrates and starchy foods as well as the mechanisms involved. Stomach cancer develops very slowly, and may take years before it produces symptoms. In the early curable stage, the symptoms rarely worry the patient and so medical advice is often sought too late. Most importantly, early symptoms of stomach cancer including a vague upper abdominal heaviness after a meal, loss of appetite, a significant weight loss and vomiting needs proper attention for early diagnosis and treatment. In conclusion, immunohistochemistry and PCR-SSCP are simple and reliable techniques to screen the alteration in p53 gene but the present result also cautions against the assumption that p53 overexpression is always associated with a gene mutation and there may be other mechanisms responsible for stabilization and accumulation of p53 protein in the nucleus.

The prognostic significance of p53 is uncertain; its role in diagnosis is promising but yet to be developed. However, there is no doubt about the central role of p53 in the development of gastric cancer and one of the main questions to be answered is: Why do a significant proportion of gastric tumors seen to have normal p53? The answer may come from intensive study of the p53 pathway rather than of p53 in isolation, as it is conceivable that all malignant tumors have a defect somewhere along its pathway. When the p53 pathway and its defects are fully understood, the therapy for gastric cancer based on its molecular and genetic profile will be a practical strategy.