Brief Reports

Changes of p53 protein blood level in esophageal cancer patients and normal subjects from a high incidence area in Henan, China *

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Esophageal cancer remains the leading cause of cancer-related death. Previous studies by us and others indicated that esophageal carcinogenesis is a multiple stage process. Abnormal cell hyperproliferation may be an early indicator for esophageal carcinogenesis. Although the molecular basis for esophageal carcinogenesis is still poorly understood, the recent studies showed that p53 protein accumulation and p53 gene mutation occur more frequently in the esophageal precancerous and cancerous lesions from the subjects at high incidence areas for esophageal cancer in Henan, China. The frequency of p53 protein accumulation and p53 gene mutation increased as the lesions progressed to cancer[1-4]. Therefore, it is much desirable to correlate the p53 protein changes in blood from esophageal cancer patients and normal subjects in this area. The present study was undertaken to further characterize the changes of p53 protein blood level in patients with esophageal cancer and to correlate the changes with those with normal esophageal epithelium.

MATERIALS AND METHODS

Subjects

All the 31 subjects were from Linzhou City (originally known as Linxian), Henan Province, a high incidence area for esophageal cancer. Histopathological examination showed that 20 subjects had primary esophageal squamous cell carcinoma and 11 had normal squamous epithelium. None of the cancer patients had received any chemotherapy or radiation therapy before operation.

Blood collection

Ten ml blood was obtained from each subject with empty stomach. The whole blood was centrifuged, compartmentalized and preserved in liquid nitrogen for p53 protein analysis.

p53 protein analysis with ELISA

Reagent. ELISA Kit for p53 protein of both wild and mutated type (Oncogene Science, Inc., USA); normal serum of rat; anti-rabbit IgG labeled by peroxidase; ELISA panel (96 wells) coated by monoclonal antibody of p53 protein (PAb1801); labeled p53 protein; autonomic ELISA counter (Fisher Co., USA); buffer solution (pH 7.4).

Procedure. The main procedures are based on the method provided by the Oncogene Science Inc. to define the standard curve of p53; diluted p53 to six different concentrations, the serum was diluted to 1: 10 with buffered solution; added 100 μl normal mouse serum in each well on the ELISA panel to block non-specific reaction; added 100 μl working solution of p53 and diluted serum in the well, and incubated for 2 hours under roomtemperature, washed with buffer solution for three times, then added sheep-anti-rabbit IgG labeled by peroxidase, incubated for one hour under room temperature, washed with buffer solution for three times, the substrate was added and incubated for 30 minutes.

RESULTS AND DISCUSSIONS

Our study showed that the mean value of p53 protein in the serum of 11 normal cases and 20 cases of esophageal cancer were 0.15 μg/L±0.09 μg/L ($\bar{x}\pm s$) and 0.23 μg/L±0.04 μg/L ($\bar{x}\pm s$) respectively. The p53 protein level in the serum of esophageal cancer patients was significantly higher than that of normal group from the same area($P<0.05$). These results were consistent with the previous observation of the high frequency of p53 protein accumulation in the early stage of esophageal carcinogenesis, suggesting that the changes of protein level in the blood may be a sensitive indicator for molecular changes in tissue

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level. Although the molecular basis for these changes was still unclear, it might be a promising circulating biomarker for esophageal carcinogenesis. Considering the fact that the esophageal and gastric cardia cancer are the most frequent tumor in Linzhou City, the changes of p53 protein blood level observed in these patients may reflect the actual response to esophageal and gastric cardia carcinogenesis. Recent studies have indicated that circulating p53 protein detected with ELISA method was mostly mutate type. Further studies should be undertaken to determine the levels of two types of p53 proteins to elucidate the biological significance of p53 gene alteration in esophageal carcinogenesis. It is also worthy to correlate the changes of p53 protein in blood and tissue during the multiple stage of esophageal carcinogenesis through long-term follow-up studies.

It is worth note that serum p53 protein was detected in the 11 cases with normal esophageal epithelium, which is consistent with the previous results that the accumulation of p53 protein and p53 gene mutation occurred in the nearly normal esophageal epithelium. Long-term follow-up studies for these subjects may not only shed a light on the mechanism of esophageal carcinogenesis, but also be of great potential for clinical application in early diagnosis and prevention of esophageal cancer.

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