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SHORT COMMUNICATION

Prevalence and Clinical Relevance of Serum Anti-p53 Antibodies in Patients with Cholangiocarcinoma

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SUMMARY Cholangiocarcinoma (CCA) constitutes carcinoma of the bile duct found at a high prevalence in northeastern Thailand. In the present study, we examined the sera of altogether 82 Thai CCA patients for the presence of anti-p53 antibodies in order to investigate a role of the tumor suppressor gene, *p53* in the carcinogenesis. Our results revealed anti-p53 antibodies in 7.3% of the cases tested, which conforms to the prevalence rate of *p53* gene mutation recently reported at 5% among Thai patients. With limited number of the patients, anti-p53 antibodies were rapidly detected more frequently among patients with peripheral tumors than those with central tumors. However, further studies is required to establish significance and prognostic value of the antibodies in the context of CCA.

Cholangiocarcinoma (CCA), a carcinoma of the bile duct, is common among inhabitants of northeastern part of Thailand where infection with the liver fluke, *Opisthorchis viverrini*, is endemic.[1] It is estimated that 70% of the population in this region have been infected with the parasite and the incidence of CCA is believed to be at least 50 times higher than in Western countries.[2] Considerable evidence gathered from epidemiological data and experimental animal models have supported the notion that prolonged liver fluke infestation through consumption of uncooked fish containing metacercariae of the parasite and high contents of nitrosamine compounds in fermented salted fish which is the main diet in this region, are the two factors mainly accountable for such a high incidence of CCA.[3-4] In this two-stage model of carcinogenesis, nitrosamines are considered to act as initiation factors, exerting carcinogenic effects, whereas the liver flukes are assumed to play a strong promoting role.[6] Despite the present knowledge as to its etiologic factors, the cellular and molecular pathogenesis of CCA is still unclear.

There has been increasing evidence that carcinogenesis eventually results from the accumulation of molecular abnormalities affecting both oncogenes and tumor suppressor genes.[7] Among the available candidates, abnormalities in *p53* gene have been those most extensively studied in that their alterations appear to play an important part in the pathogenesis of approximately 50% of human cancers,[7] This gene encodes a 53 kDa nuclear phosphoprotein with a short half-life that binds to DNA and can both induce cell cycle arrest and promote apoptosis[8]. Thus, mutations of *p53* could inhibit apoptosis after an irreparable genotoxic event and permit the survival of mutated cells that may lead to malignant transformation through a multi-step process.[9]

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The incidence of *p53* gene mutations in CCA has been shown to differ among different etiological backgrounds, ranging from 5 to 85%.[10-15] In the present study, we examined the frequency of serum anti-p53 antibodies, known to be products of the host immune response to mutated p53 protein,[16] in 82 Thai patients with CCA. Moreover, the relation between the development of such antibodies and other clinical variables was evaluated.

MATERIALS AND METHODS

Serum samples of hospitalized patients with CCA were collected from two hospitals: 11 cases from Chulalongkorn University Hospital, Bangkok, Thailand, and 71 cases from Udonthani Hospital, Udonthani, northeastern Thailand. Among these, 47 cases were classified as hilar type and the remaining 35 cases as peripheral type according to the locations of the tumors along the biliary tree.[17] They comprised 51 males and 31 females with their age ranging from 41 to 93 years (mean 63.5 years). The peripheral type CCA was diagnosed based on liver tumor features detected by ultrasound/CT scan and in some cases were confirmed by histology. Criteria for diagnosis of the hilar type included characteristic findings on ultrasound/CT scan or by cholangiography.[1] Demographic data such as sex and age, and clinical data including liver function tests, tumor size, tumor location, lymph node involvement and extrahepatobiliary metastasis were recorded. All patients were informed as to the objective of the study, and subsequently provided their consent. Blood samples were obtained at the initial presentation, individual sera were separated by centrifugation and stored at –20°C until test.

The patients' sera were subjected to ELISA (Pharma Cell, Paris, France) according to the manufacturer's specifications.

Data were presented as percentage, mean and standard deviation. Comparisons between groups depending on presence or absence of anti-p53 antibodies were performed by the χ^2 or Fisher's exact test for categorical variables and by the Mann-Whitney test, Fisher's exact or unpaired t test when appropriate for quantitative variables. The p values below 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Using the p53 antibody titer ≥ 1.1 U/ml as a cut-off point between positive and negative results, a positive reaction to p53 was detected in the sera of 7.3% (6/82) of CCA patients (ranging from 1.1 to 14.1 U/ml). Comparison between CCA patients positive for anti-p53 antibodies with those negative, it was found that among the former men by far outnumbered women, the peripheral type of CCA was more prominent and the mean serum albumin was significantly higher. There were no differences between groups with regard to other factors including age of the patients, tumor size, lymph node metastasis and the remaining biochemical parameters of the liver function test (Table 1).

The incidence of *p53* gene mutations in CCA displays a variability between studies, ranging from 5% to 85%[10-15]. These discrepancies between studies might be attributable to differences in geographical and environmental factors responsible for inducing tumor pathogenesis.

Conventionally, the detection of *p53* mutations has been performed by DNA sequence or by immunohistochemical analysis of abnormal p53 protein accumulation in cancerous cells, both of which require tumor tissue obtained from either needle biopsy or surgical specimens[18]. Only recently, serum anti-p53 antibodies have been demonstrated to be products of the host immune response to mutated p53 protein detectable in sera of patients with a variety of cancers[16]. In most cases, such antibodies have been considered specific to either p53 accumulation or *p53* mutation.[16,19,20] Moreover, detectable serum anti-p53 antibodies seem to indicate a more aggressive behavior of certain tumor types such as breast, gastric and colorectal cancers.[21-23]

To our knowledge, the prevalence and clinical significance of serum anti-p53 antibodies have never been studied among patients with CCA. In the present study, elevated p53 antibody levels were detected in 6 of 82 cases (7.3%). This prevalence rate is in accordance with the mutation rate of the *p53* gene, which has been reported to amount to 5% in a recent study on Thai patients.[13] Conversely, its frequency was much lower than that reported in another study demonstrating a similar incidence of mutations in this gene among Japanese and Thai CCA patients (33% and 35%, respectively).[10] Nonetheless, it is noteworthy that many tumors bearing *p53* gene mutations do not always elicit antibody responses. For example, only tumors with *p53* gene missense mutations, as opposed to stop, splice/stop, splice or frame-

Table 1 Clinical features of CCA patients with respect to serum anti-p53 antibodies

Characteristics	Anti-p53 positive (n = 6)	Anti-p53 negative (n = 76)	*P* value
Age (years)	58.0 ± 10.1	63.9 ± 11.5	NS
Sex (male/female)	6/0	42/34	0.03
Tumor type (central/peripheral)	1/5	46/30	0.02
Maximum tumor size (cm.)	8.0 ± 1.4	8.4 ± 2.1	NS
Lymph node metastasis (+/-)	1/5	13/63	NS
Biochemical liver function tests			
Total Bilirubin (mg/dl)	4.4 ± 7.1	11.2 ± 13.2	NS
Alkaline phosphatase (IU/l)	516.7 ± 226.6	693.1 ± 634.1	NS
AST (IU/l)	110.8 ± 45.4	107.5 ± 98.3	NS
ALT (IU/l)	98.3 ± 59.8	87.0 ± 147.9	NS
Albumin (g/dl)	3.9 ± 0.8	3.2 ± 0.7	0.02
Prothrombin time (seconds)	13.1 ± 2.1	16.2 ± 8.6	NS

shift mutations, are able to induce antibodies, as the amount of protein produced in the latter is insufficient to elicit an immune reaction.[24] Since in the present study we did not examine *p53* gene mutations occurring in tissue specimens, we could not establish a correlation between the presence of p53 antibodies and mutations in the *p53* gene. In this regard, additional studies are still required to validate such association.

Analysis of clinical parameters demonstrated a relation between the presence of p53 antibodies and tumor location, as peripheral CCA was more frequently found than the hilar type. These findings were similar to those of Kang *et al.*[15] whose data showed that *p53* gene mutations were prominent in the peripheral type of CCA, as well as among certain gross tumor types such as the mass-forming type. The discrepancies observed in clinical presentations and prognoses between types of CCA could provide an explanation why *p53* gene mutations, which usually occur during later stages, are more prevalent in the peripheral type.

The prognosis of CCA is generally poor due to an advanced stage at presentation. Unfortunately, in the present study we could not evaluate the survival time of the majority of patients, as most cases were lost to follow-up after the diagnosis. However, it is tempting to speculate that the presence of anti-p53 antibodies in serum might not be associated with poor prognosis as patients in both groups (positive and negative anti-p53 antibodies) were comparable regarding clinical stages and, more importantly, none of the patients did undergo surgical resection, as yet the method entailing the greatest chance for prolonged survival[25]. Similarly, recent data have also failed to establish the prognostic significance of *p53* mutations in patients with CCA.[26,27]

In conclusion, we observed a low prevalence of serum anti-p53 antibodies in Thai patients with CCA. The presence of such antibodies appears predominant in CCA of the peripheral type. However in the present study, the validity of serum anti-p53 antibodies as prognostic factors for CCA has not been established. Comparably results of anti-p53 antibodies have been reported earlier, in a study on patients with head and neck cancer, that the antibodies are not suitable marker for malignancy.[28] In contrast, another group studied patients with lung cancer found the presence of serum anti-p53 antibodies to be closely associated with malignant pleural effusions.[29] Therefore, further studies aimed at elucidating the correlation between serum anti-p53 antibodies and gene mutations in tumorous tissue, as well as their prognostic value, are required.

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