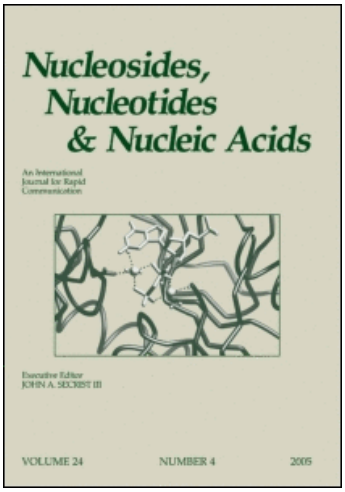

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Thymidine Kinase 1 is a Potential Marker for Prognosis and Monitoring the Response to Treatment of Patients with Breast, Lung, and Esophageal Cancer and Non-Hodgkin's Lymphoma

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THYMIDINE KINASE 1 IS A POTENTIAL MARKER FOR PROGNOSIS AND MONITORING THE RESPONSE TO TREATMENT OF PATIENTS WITH BREAST, LUNG, AND ESOPHAGEAL CANCER AND NON-HODGKIN'S LYMPHOMA

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□ *Thymidine kinase 1 (TK1) is converting thymidine to thymidine monophosphate, and is related to DNA replication and cell proliferation. The use of the TK1 protein levels as a proliferation marker in malignancies is here summarized. TK1 protein in serum (STK1p) and TK1 expression in tissues were determined by a chemoluminescent dot blot assay and by immunohistochemistry staining, respectively. The expression of TK1 in tumor tissues correlated to pathological stages and clinical grades of carcinomas (ca) of esophagus, lung and in premalignancy of breast ductal ca. STK1p could monitor the out-come of tumor therapy by being correlated to remission [breast ca, non-Hodgkin's lymphoma], relapse [breast ca] and to survival [non-Hodgkin's lymphoma] of patients. In a health screening study of 12,641 persons, STK1p seemed to predict the risk of development of neoplasia related diseases at early stage.*

Keywords Thymidine kinase 1; serum; immunohistochemistry; malignancies

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INTRODUCTION

Thymidine kinase 1 (TK1) is a useful tumor proliferation marker, since its level is highly proliferation dependent. Therefore, the activity of TK in serum (STKa) has been used as a marker for malignancy since 1984. Although STKa is useful in patients with leukemia and lymphoma it is not in patients with solid tumors. Poly- and monoclonal antibodies against human TK1 have been produced[1,2] for the determination of TK1 protein levels in serum (STK1p), based on a sensitive enhanced chemoluminescent (ECL) dot blot assay.[3] STK1p values in 2,550 patients with 10 different types of malignancies and in health screening of 20,853 persons have been determined since 2005, using TK1 chicken polyclonal and mouse monoclonal antibodies (SSTK Inc., Shenzhen, China). Here, we summarize some of these results.

MATERIAL AND METHODS

Patients

For this study, patients with various types of carcinomas (breast, lung [NSCLC], esophagus) and of non-Hodgkin's lymphoma were examined at several university hospitals in Shenzhen, Fuzhou, Hangzhou, and Shanghai, and healthy persons attending health centers in Changsha and Hangzhou, China, since 2005 (Table 1). The pathological staging of tumors was determined according to the AJCC Cancer Staging System and grades according to the World Health Organization (WHO) tumor grading system.[4] All patients gave informed consent, according to the Helsinki Declaration 1983 of the World Medical Association.

STK1p Levels

The STK1p was determined by the commercial TK1 Diagnostic Kit (SSTK Inc., Shenzhen, China), previously described by He et al.[3] An amount of 3 μl of serum from non-heparinized blood was directly applied to nitrocellulose membranes. Then primary TK1 IgY antibody was added, followed by biotinylated secondary antibody, HRP streptavidin, and ECL detection.[3] The intensities of the spots on the membranes were determined by CIS-1 imaging (SSTK Inc., Shenzhen, China). From the intensity of a TK1 standard of known concentrations, the concentration of serum TK1 was calculated and expressed as pM. The accuracy of the dot blot assay is 4–6% and the sensitivity less than 0.3 pM.[3]

Immunohistochemistry

Immunohistochemistry was carried out with the Dako EnVision System (DAKO, Copenhagen, Denmark) as described.[5] In brief, sections were

incubated for 5 minutes using 3% H_2O_2 in order to block the endogenous peroxidase. Nonspecific binding sites were blocked by incubating the sections with blocking buffer including in the EnVision Kit (DAKO, Copenhagen, Denmark). After incubation with the anti-TK1 mAb (800 X PBS dilution in 1 mg/ml, SSTK Inc, Shenzhen, China) for 2 hours at room temperature, the sections were rinsed in PBS. EnVision conjugate (DAKO, Copenhagen, Denmark) was added and then incubated for 40 minutes at room temperature. Diamino-benzidine (DAB) was used as a chromogen. The anti-TK1 mAb was produced in mouse by immunization of a 31 mer peptide corresponding to the C-terminal end of TK1.[5]

Statistical Analysis

The mean values of STK1 were calculated by a mean ± SD program. When calculated the statistical significance between different groups of patients, student's t-test and chi-square were used. The significance of the 5-year survival was calculated by log-rank test. Differences were considered to be significant when the *p*-value was less than 0.05.

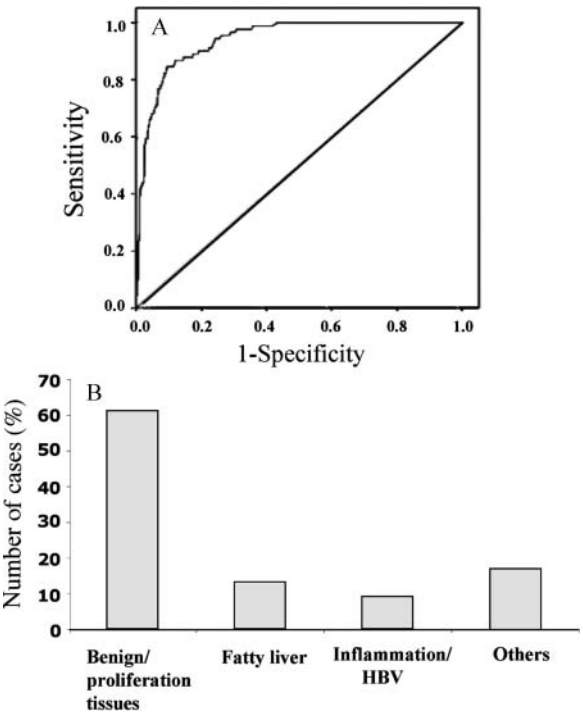

FIGURE 1 A) Relationships between sensitivity and specificity of tumor patients (n = 224) and healthy persons (n = 761). B) Anomalies in persons with STK1p values above 2.0 pM (n = 54) diagnosed by clinical methods including imaging.

RESULTS AND DISCUSSION

Health Screening

The Receiver Operating Characteristic (ROC) value of the STK1p dot blot assay was 0.941, showing high reliability (Figure 1A). In a health screening study with 11,278 persons only 0.5% were STK1p positive (>2.0 pM), of which 80% had some form of malignancy related diseases (Figure 1B). The

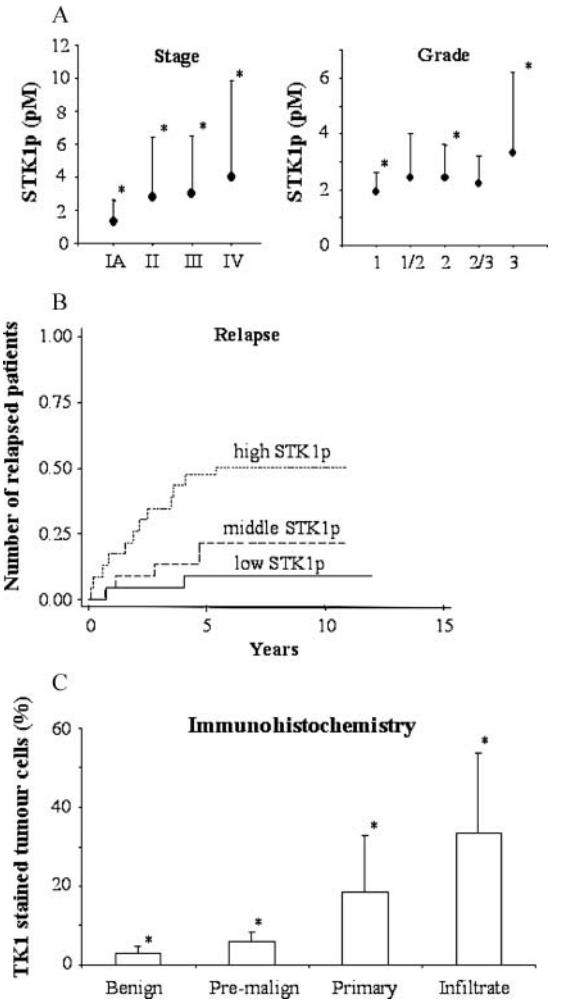

FIGURE 2 A) STK1 levels in relation to stage of esophagus carcinoma patients (n = 129) and to grade of lung carcinoma patients [NSCL] (n = 155) in serum samples. B) STK1p levels in serum samples at 3 months after surgery of breast carcinoma patients samples (n = 67) in relation to relapse. The STK1p value of each patient at 3 months after surgery was related to the STK1p value at surgery and expressed in percentage. High value >110%, middle value = 78–110%, low value = <78%. C) Number of TK1 positive immunohistologically stained tumor cells in breast ductal carcinoma patients (n = 88). The tumor sections were collected from paraffin blocks. $^*p < 0.05$.

number of persons with breast and prostate proliferation anomalies were significantly higher in STK1p positive persons compared to STK1p negative ones (<2.0 pM; $p < 0.05$).[6] Although the number of persons in this study is not high enough to draw final conclusions about the use of STK1p in health screening, results from an additional health screening of 8,869 persons[7] and preliminary results from a health screening of 10,578 persons (Fuzhou, China, 2008–2009, personal communication), showed similar results. Thus, STK1p may be a reliable tumor proliferating marker, enabling early discovery of risk for cancer development in premalignancy/early malignancy.

Prognosis

STK1p values correlated to the pathological stages (esophageal ca) and clinical grades (lung ca [NSCL]) (Figure 2A). There was also a correlation between the STK1p value 3 months after surgery, and a risk of breast ca relapse (follow-up study of 11 years; Figure 2B). There was a 6.1 higher risk to have relapse with high STK1p values ($p = 0.004$).[8] Although these breast ca patients belonged to a low risk group, the STK1p value was able to predict the risk of developing relapse already at 3 months after surgery. This is in agreement with a recent study on breast ca patients where the activity of

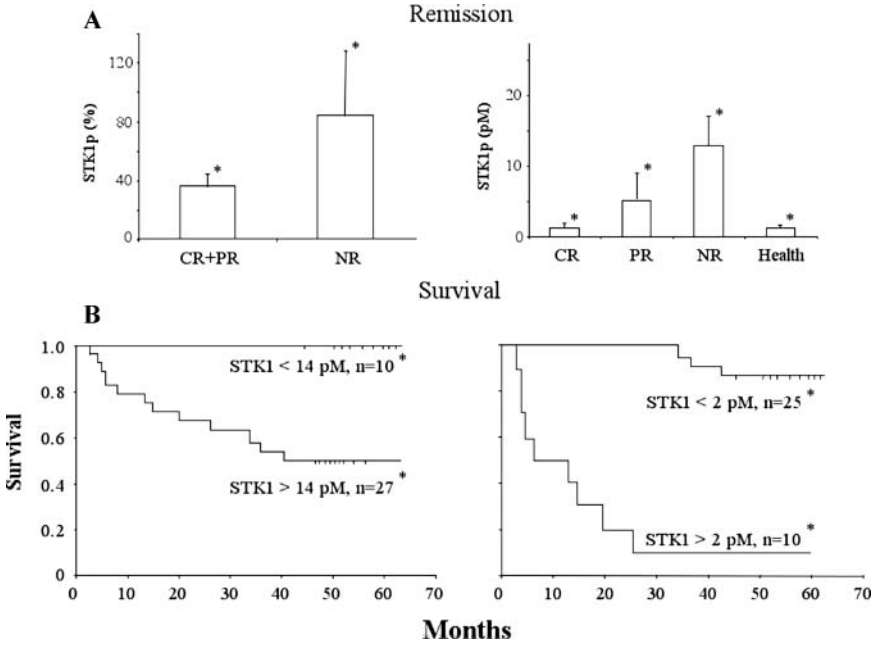

FIGURE 3 A) STK1p levels in relation to remission (CR = complete remission, PR = partial remission, NR = no remission) of breast (left, n = 19) and non-Hodgkin's lymphoma (right, n = 57) patients. B) STK1p values in relation to 5-year survival of non-Hodgkin's lymphoma patients (n = 37); STK1p values before start of treatment (left) and 28 days after start of treatment (right). *$p < 0.01$.

TABLE 1 Characterization of tumor patients and healthy persons

Types	Number of cases
ROC-values:	esophagus = 33;cervical = 32; lung = 25;ovarian = 24;esophagus
Tumor patients	= 22; gastric = 16; breast = 14; colon = 10; rectum = 9; liver = 7; lymphoma = 7; another's = 25.
Healthy persons	761
Health screening:	
TK1-positive (>2.0 pM)	54
TK1-negative (<2.0 pM):	11,224
Prognosis:	
Stage, esophagus ca	129
Grade, lung ca (NSCL)	155
Relapse, breast ca	69
Immunohistochemistry, breast ca.	88
Remission:	
Breast ca	19
Non-Hodgkin's lymphoma	37
Survival:	
Non-Hodgkin's lymphoma	37

TK1 in serum (STK1a) was determined, showing that STK1a was able to predict relapse 9 months before appearance of relapse.[9] The expression of TK1 in breast ductal ca was significantly different between various types of benign/pre- and malignant types of breast tissues (TK1 expression in benign < premalignant < primary < infiltrate) (Figure 2C).[5] Identification of patients with premalignancy is of importance for a positive prognosis. The observation that TK1 expression in premalignant patients significantly differ from patients with malignant and benign diseases increases the possibility of timely treatments at early stage of malignancy with minimal access surgery, improving the survival rates.

Tumor Therapy

STK1p values correlated to remission after surgery (breast carcinoma, esophagus, Figure 3A, left) and after chemotherapy (non-Hodgkin's lymphoma, Figure 3A, right). STK1p values could also be used to predict the 5-year survival of non-Hodgkin's lymphoma patients, when determined before start of chemotherapy (Figure 3B, left) and 28 days after start of the chemotherapy (Figure 3B, right). In a previous study, the intracellular expressions of TK1 in human breast tumours were also found to predict the survival of breast cancer patients.[10]

CONCLUSION

STK1p determinations in tumor tissues and sera provide accurate information about the prognosis of cancer patients, for monitoring outcome of tumour therapy and for early discover of proliferation related diseases including cancer.

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