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ORIGINAL ARTICLE

Serum thymidine kinase 1 correlates to clinical stages and clinical reactions and monitors the outcome of therapy of 1,247 cancer patients in routine clinical settings

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Abstract
Background Thymidine kinase 1 in serum (STK1) has been found to be a reliable proliferation marker in clinical trials. In this study, we examined the significance of STK1 in routine clinical settings.
Methods The concentration of STK1 was determined by a sensitive dot blot ECL assay. The STK1 value was correlated to clinical stage and reactions and used for monitoring the outcome of surgery and/or multidrug chemotherapy of 1,247 patients with five different types of carcinomas (lung, esophagus, gastric, head and neck, and thyroid) in routine clinical settings.
Results The STK1 values correlated with the clinical stage in patients with lung, esophagus, thyroid, and gastric carcinomas. After treatment, STK1 declined in all tumor groups after treatments ($P < 0.01$). The STK1 was low (<2 pM) or decreasing during treatment in patients with clinical reactions of complete response (CR) or partial response (PR), but high (>2 pM) or increasing in patients with stable disease (SD) or progressive disease (PD), some of them showing metastasis. STK1 also reflected the differences in clinical reactions when surgery and chemotherapy were compared.
Conclusion We concluded that the concentration of TK1 in serum correlates to clinical stages and clinical reactions and monitors the effect of tumor therapies, not only in controlled clinical trials, but also in routine clinical settings.

Keywords Serum thymidine kinase 1 (STK1) · Lung carcinoma · Esophagus carcinoma · Gastric carcinoma · Head and neck carcinoma · Thyroid carcinoma · Tumor therapy

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Introduction

Numbers of potential serum markers have been investigated for detection of invisible tumors. Some of them, for example, carcinoembryonic antigen (CEA), CA19.9 [1–3], CA125 [4], alpha-fetoprotein (AFP) [5], and prostate-specific antigen (PSA) [6], are in routine clinical use today after being tested in controlled clinical trials. CA15-3 is useful in patients with metastatic breast cancer unassailable for response to systemic therapy [4–7]. The combination of TPS, CA15-3, and IGFBP-3 increases the sensitivity of these markers to 85% and thus improve the predictive ability [8]. CA125 level is associated with a worse prognosis, mainly related to relapse [4]. Serum CA125 is an imperative indicator for malignancies of ovary carcinoma. In 2006, the American Society of Clinical Oncology (ASCO) recommended that CEA could be used for colorectal cancer preoperatively [9], assisting in staging and surgical planning. Although showing promising results

from clinical trials and clinical routine settings, the predictive role of serum tumor markers for response to anticancer therapy is still controversial, and accurate attention must be paid to individual characteristics [6]. Furthermore, multivariate analysis of different studies show that serum tumor markers may fluctuate more than changes detected with imaging techniques.

Thymidine kinase 1 (TK1), a pyrimidine metabolic pathway enzyme involved in salvage DNA synthesis, is cell-cycle dependent and thus a proliferation marker. The serum thymidine kinase 1 (STK1) level in malignant tissues is proportional to cellular proliferating rates [10–13]. Thymidine kinase activity has been used in serum and cytosol fractions of tissues as a proliferation marker since 1980. TK1 activity is almost undetectable in the serum of healthy persons [14–16], but it increases to different levels in patients with malignancies depending on tumor type, fast or slow growth rate [17], and stage [16]. In a clinical controlled study of 1,692 breast cancer patients [18], TK1 activity in the cytosol correlated to a shorter survival as well as a poor outcome of endocrine treatment (tamoxifen) [19]. Recently, commercially available anti-TK1 antibodies, measuring the concentration of TK1 in serum instead of its activity, have provided an attractive alternative in clinical cancer application of serum markers. In patients with solid tumors, the concentration of TK1 in serum (STK1), as measured by anti-TK1 antibodies, is a more sensitive and reliable marker as compared to the TK activity [20, 21]. In controlled clinical studies, the STK1 level correlated with relapse (breast carcinoma) [20] and outcome of surgery (bladder carcinoma) [22]. However, the serological TK activity and CA 15-3 did not correlate with relapse in breast carcinoma patients [20]. Thus, controlled clinical studies show that STK1 is a useful marker for relapse and monitoring tumor therapy. However, in routine clinical work it is not always possible to follow recommendations from controlled clinical trials for reasons of the need to change treatment individually from time to time, i.e., type of treatment, dosages, and time schedule, which makes the prognosis of therapies less predictable. In this study, we examined the correlation of STK1 to clinical stages and clinical reactions [complete response (CR), partial response (PR), stable disease (SD), and progressive disease (PD)] and with the outcome of tumor therapy in routine clinical work.

Patients and methods

Patients

Serum samples of 1,247 patients with carcinomas of lung (no specific type of carcinoma was give in patient files; $n = 303$, mean age, 57 ± 12 years, median, 58 years, 70% men, 30% women); esophagus ($n = 363$, mean age, 57 ± 10 years, median, 56 years, 74% men, 26% women); gastric ($n = 69$, mean age, 57 ± 11 years, median, 57 years, 75% men, 25% women); head and neck (tongue cancer, pharynx cancer, parotid gland, larynx, nasopharynx, tonsil cancer, facial cancer, gum cancer; $n = 138$, mean age, 46 ± 17 years, median, 45 years, 79% men, 21% women), and thyroid ($n = 374$, mean age, 44 ± 13 years, median, 44 years, 18% men, 82% women) were collected from unselected cases in routine clinical settings, including pre- and postoperation, and/or chemotherapy in combination with surgery, at the Fujian Cancer Hospital Fujian Province, China, during 2005–2007. All patients had histologically diagnosed malignant tumor as prospectively evaluated. Some of the malignant patients had tumor records with clinical stage and type of pathology (adenocarcinoma, squamous) according to the American Joint Committee on Cancer (AJCC) cancer staging [23]. Persons ($n = 451$) confirmed not to have any illness or diseases, i.e., healthy persons, were used as negative controls (mean age, 43 ± 11 years, range, 30–70 years, 58% men, 42% women). The number of patients in the various groups studied is summarized in the Appendix (see Table 8).

Treatments

The tumors of the patients were treated by surgery, multidrug chemotherapy, or a combination of surgery and multidrug chemotherapy. The surgery was carried out according to normal procedures. The clinical reactions were determined by ultrasound and/or computed tomography (CT) techniques. The chemotherapies for the various types of malignancies followed recommended strategies, as follows. Lung carcinoma: EP program (Ito-Park glycosides 100 mg/m^2 and cisplatin 75–100 mg/m^2, in 3 weeks × 3); GC program (gemcitabine 1,000 mg/m^2 and cisplatin 75–100 mg/m^2, in 3 weeks × 3). Esophagus cancer: PBV program (cisplatin 50 mg/m^2, bleomycin 10 mg/m^2, and vindesine 3 mg/m^2, in 3 weeks × 3). Gastric cancer: ECF program (epirubicin 60 mg/m^2, cisplatin 50 mg/m^2, and fluorouracil 2 g/m^2, in 2 weeks × 3). Head and neck cancer: PC program (paclitaxel 135–175 mg/m^2 and cisplatin 80 mg/m^2, in 3 weeks × 3). Patients with thyroid carcinoma received only surgery.

Follow-up

In 38 patients with different types of carcinomas (lung, $n = 12$, follow-up 11 months; esophagus, $n = 23$, follow-up 5 months; gastric, $n = 5$, follow-up 5 months), the clinical reactions (CR, complete remission; PR, partial remission; SP, stable disease; PD, progressive disease,

following RECIST criteria) of individual patients were determined after treatments and compared to STK1 values. The clinical reactions of another group of patients ($n = 223$) were also determined and compared to the STK1 values.

The present study of serum TK1 was conducted in accordance with the Helsinki Declaration of 1983. All persons gave their informed consent before their inclusion in this study.

ECL dot blot assay of STK1

STK1 was analyzed before and after treatment by an ECL dot blot assay. The procedure was performed according to the manufacturer's protocol (commercial kit; SSTK, Shenzhen, China) as described elsewhere [24]. The sensitivity of the assay was 0.1 pM. All experiments were done in blind. The TK1 antibody has been characterized by biochemistry [25], showing one single band in Western blots of serum of patients with gastric carcinoma, corresponding to the native form of TK1. No TK1 band was found in TK1-negative cells and in serum from healthy persons with no detectable level of TK1 [25]. The receiver operating characteristic value (ROC value) of the dot blot TK1 assay was 0.941 [24].

Statistical calculations

The mean values of STK1 were calculated by a mean ± standard deviation (SD) program. When calculating statistical significance between different groups of patients, Student's t test and compare groups–proportions–chi-square test were used (Analyse-it software, UK). Differences were considered to be significant when the P value was less than 0.05.

Results

The number of patients in the various groups analyzed is shown in the Appendix (see Table 8).

STK1 in relationship to pre-treated patients

STK1 level of healthy persons, untreated patients with malignancies, and patients with benign tumors

The mean STK1 value of the healthy persons was 0.4 ± 0.3 pM, statistically significantly ($P < 0.001$) lower as compared to the untreated patients with malignancies with different types of carcinomas (Table 1). The mean STK1 value of patients with benign tumor types of the head and neck (1.4 ± 2.0, $n = 65$) was significantly higher ($P < 0.001$) compared to the healthy persons, but significantly lower than that of head and neck carcinoma patients ($P < 0.001$).

Table 1 Serum thymidine kinase 1 (STK1) values of healthy persons and of untreated patients with different types of malignancies

STK1 (pM)	Healthy	Lung	Esophagus	Gastric	Head and neck	Thyroid
Mean	0.4	2.8	3.1	1.7	3.0	4.5
SD	0.3	3.9	4.4	1.3	4.5	6.8
n	451	238	267	37	115	327

Mean values ± standard deviation (SD)

STK1 values in relationship to clinical stage and pathological type

The STK1 values were significantly correlated with clinical stage of carcinoma of lung (IA → IV, $P = 0.013$) (Fig. 1a), esophagus (Ia → III and IV, $P < 0.032$) (Fig. 1b), and thyroid (I → IV, $P = 0.013$) (Fig. 1d), with a trend of higher STK1 values in patients with gastric carcinoma (Fig. 1c). No data of clinical stages were given for the head and neck carcinoma patients. There were no significant differences in mean STK1 values between adenocarcinoma (AC) and squamous (SCC) types of tumors (AC: 3.9 ± 3.9 pM, $n = 28$; SCC: 4.5 ± 6.5 pM, $n = 41$, $P = 0.601$).

STK1 in relationship to tumor size

The STK1 values of some patients correlated closely to the size of the tumors, but in other patients the STK1 values did not. To understand this discrepancy, the patients were subdivided into four main STK1/size groups: low STK1/small tumor, high STK1/small tumor, low STK1/large tumor, and high STK1/large tumor. The size discriminations of the tumors were set according to the pathology recommendations of the AJCC. The threshold for the STK1 values was set to 3.0 pM, except for the gastric carcinoma patients, which was set to 2.0 pM. The clinical stages of the individual patients within the four subgroups were also determined to judge possible differences in the clinical outcome of these subgroups. The results are shown in Fig. 2 and Table 2.

Small tumor size Among the patients studied, only patients with thyroid carcinoma showed a higher frequency of later clinical stages when the patients with small tumors (<3 cm) exhibited higher STK1 values (Fig. 2d, Table 2). However, these changes were not statistically significant (Table 2). In contrast, lung carcinoma patients with small tumors and higher STK1 values represent patients with earlier clinical stages (Fig. 2a, Table 2). Again, these

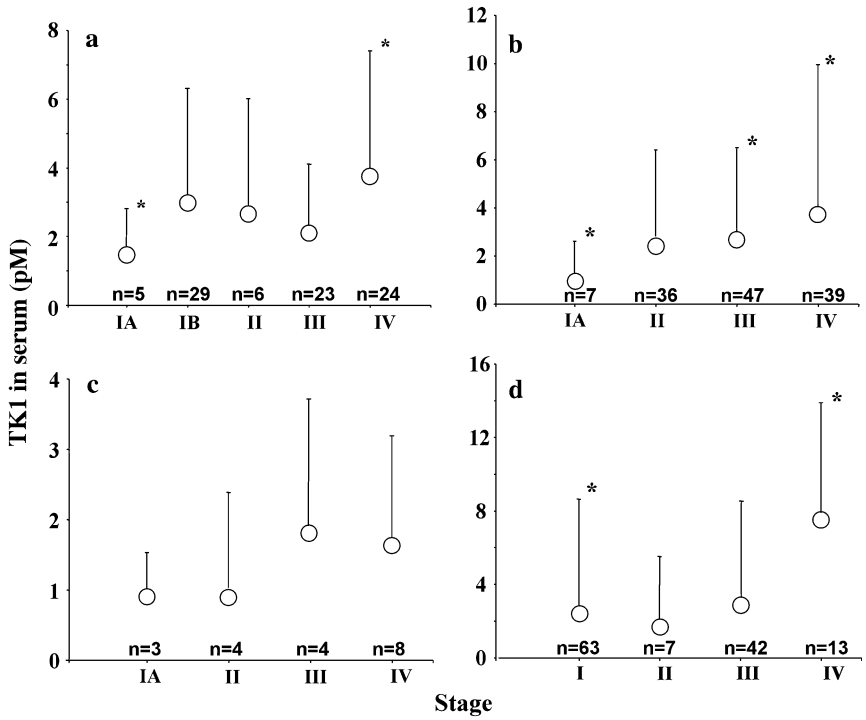

Fig. 1 Mean thymidine kinase 1 in serum (STK1) values in relationship to clinical stages of patients with lung ($n = 87$) (**a**), esophagus ($n = 129$) (**b**), gastric ($n = 19$) (**c**), and thyroid ($n = 125$) (**d**) carcinomas. *Stars* indicate statistical significance (Student's t test)

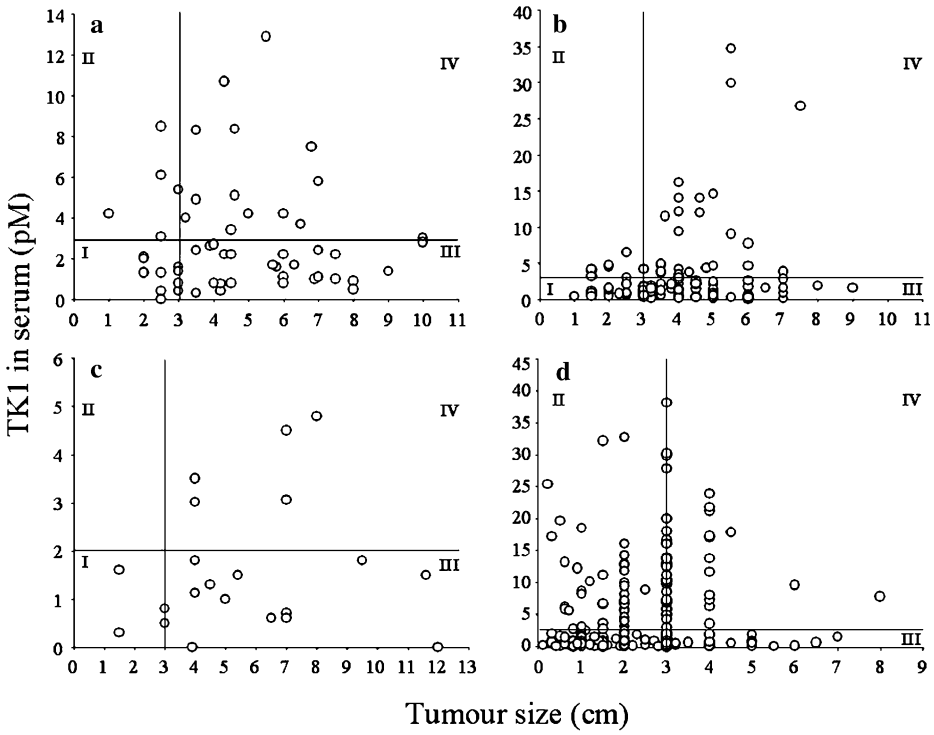

Fig. 2 Individual STK1 values in relationship to tumor size of patients with lung ($n = 59$) (**a**), esophagus ($n = 115$) (**b**), gastric ($n = 24$) (**c**), and thyroid ($n = 282$) (**d**) carcinomas. *Roman numbers* represent the various STK1/tumor subgroups: *I*, low STK1/small size; *II*, high STK1/small size; *III*, low STK1/large size; *IV*, high STK1/large size

changes were not statistically significant (Table 2). In esophagus carcinoma patients, there were no obvious differences between patients with small tumors with lower or higher STK1 values (Fig. 2b, Table 2). No data were obtained from the gastric and head and neck carcinoma patients in this respect (small tumor/low or high STK1 values).

Large tumor size In the thyroid carcinoma patients with larger tumors and higher STK1 values, there was a significantly higher frequency of patients with stage IV ($P = 0.010$), as compared to patients with lower STK1 values. In the patients with gastric carcinoma, there was also an increase in the number of patients with stage IV at higher STK1 values (from 44% to 60%), but it was not

Table 2 The various STK1/tumor size subgroups in relationship to clinical stages

Carcinoma	Low STK1/small	High STK1/small	Low STK1/large	High STK1/large
Lung	IB (30%)	**IA + IB (100%)**	**IA + IB (58%)**	**IB (33%)**
			IIA + B (8%)	IIB (20%)
	IIIA + B (60%)		IIIA (19%)	IIIA + B (27%)
	IV (10%)		IV (15%)	IV (20%)
Esophagus	IA (14%)	IA (29%)	IIA (13%)	IIA (18%)
	IIA (43%)	**IIA (42%)**	IIB (4%)	
	IIB (14%)			
	III (10%)		**III (48%)**	**III (57%)**
	IV (19%)	IV (29%)	IV (35%)	IV (25%)
Gastric	**I (50%)**	n.d.	I (12%)	II (20%)
	II (50%)		II (22%)	III (20%)
			III (22%)	**IV (60%)**
			IV (44%)	
Head and neck	n.d.	n.d.	n.d.	n.d.
Thyroid	**I (62%)**	I (40%)	**I (38%)**	I (33%)
	II (3%)		II (20%)	
	III (32%)	**III (60%)**	**III (38%)**	III (17%)
	IV (3%)		IV (4%)	**IV (50%)**

The bold text indicates the highest values

statistically different ($P = 0.751$). In the lung carcinoma patients with larger tumors and lower STK1 values, there was an unexpectedly high frequency of patients of stages I and II. In the lung carcinoma patients with larger tumors and higher STK1 values, the number of stage I patients decreased from 58% to 33%; however, this was not statistically significant ($P = 0.366$).

STK1 in relationship to patients post treatment

In this part of the study, two groups of patients were evaluated. In the first group of patients, the mean STK1 values before and after treatment were determined (Table 3). These treated patients are not a follow-up of the untreated patients (shown in Table 1), but an independent group of treated patients. In all five malignant groups of patients, the mean STK1 values decreased significantly after the treatments (surgery and/or chemotherapy; $P < 0.01$), except for the patients with gastric carcinoma.

In the second part of this study we examined whether the STK1 value could distinguish between the effects of surgery and chemotherapy alone or in combination.

Lung carcinoma

The clinical stage I–III patients received surgery + chemotherapy, whereas clinical stage IV patients only received chemotherapy (Fig. 3a). To be able to judge changes in STK1 levels after chemotherapy as compared to surgery + chemotherapy, we had to compare these with the STK1 values of untreated patients of clinical stage IV. The

Table 3 STK1 values of patients with different carcinomas after surgery and/or chemotherapy

Carcinomas	Before	After	*P* value
Lung ($n = 65$)	2.8 ± 3.9	1.6 ± 2.0	0.004
Esophagus ($n = 96$)	3.1 ± 4.4	1.6 ± 3.4	<0.001
Gastric ($n = 32$)	1.7 ± 1.3	1.3 ± 1.8	0.434
Head and neck ($n = 28$)	3.0 ± 4.5	1.1 ± 0.8	<0.001
Thyroid ($n = 47$)	4.5 ± 6.8	1.3 ± 0.9	<0.001

Numbers in parentheses represents number of patients after treatments. Data are mean values ± standard deviation (SD), before and after treatments. *P* values were obtained from Student's *t* test

STK1 values were reduced by 83% (statistically significant at $P < 0.001$) after chemotherapy. No significant difference in STK1 values was found between healthy persons and those patients receiving chemotherapy ($P = 0.088$), showing extensive decrease in STK1 values after chemotherapy. Patients receiving surgery + chemotherapy were of clinical stage I–III. When we compared the STK1 values of these patients with untreated patients with the same clinical stages (I–III), the STK1 values decreased by 37%, which was statistically not significant ($P = 0.123$).

Esophagus carcinoma

STK1 values decreased by 45% after surgery (statistically significant at $P = 0.009$). In patients receiving chemotherapy, the STK1 value decreased by 71%. However, there was no statistically significant difference between

STK1 values after surgery and after chemotherapy ($P = 0.114$) (Fig. 3b).

Head and neck carcinoma

After surgery, the STK1 values declined by 63% (statistically significant at $P < 0.001$). When patients were treated with chemotherapy, no decrease in the STK1 values was found (Fig. 3c).

STK1 in relationship to metastasis after treatment

Metastasis was reported in some of the patients with lung, esophagus, and gastric carcinoma. In esophagus and gastric carcinoma patients with metastasis, the STK1 values were not reduced so much as in patients without metastasis after treatment. No such differences were found in patients with lung carcinoma (Table 4).

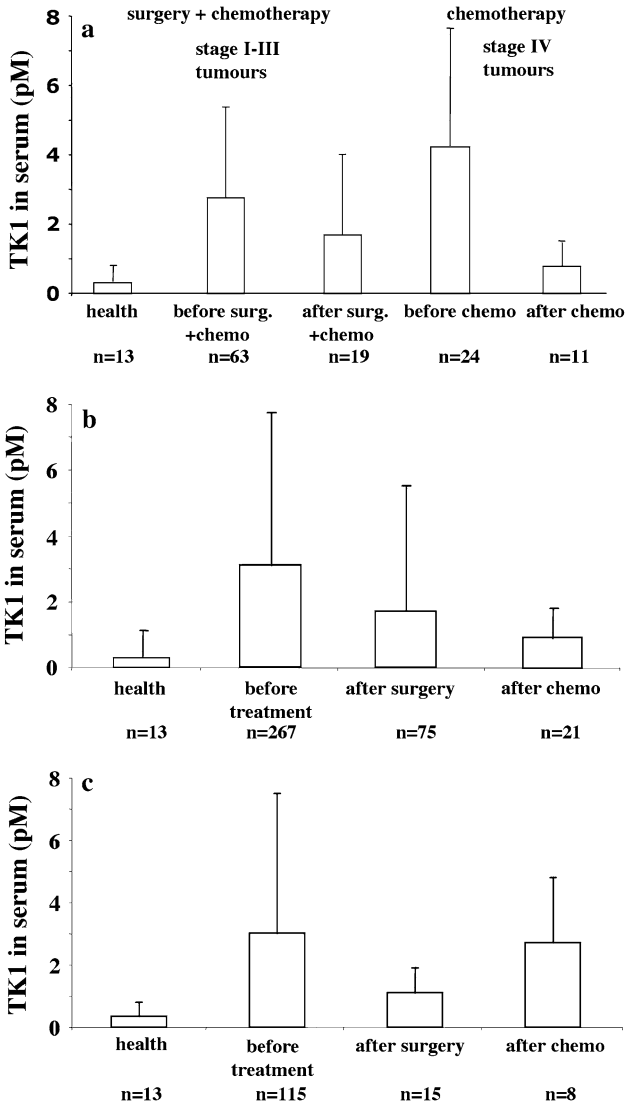

Fig. 3 TK1 in sera of healthy persons and of patients with lung (**a**), esophagus (**b**), and head and neck (**c**) carcinomas after treatments. Mean values ± standard deviation. *Chemo*, chemotherapy

STK1 in relationship to clinical reaction after tumor treatment

In a follow-up study up to 11 months after treatment of 40 patients with lung, esophagus, and gastric carcinomas, the clinical reactions (CR, PR, SD, PD) were examined in individual patients. These results were then correlated to the changes in STK1 values before and after treatments. In more than 70% of the cases, there was a close correlation between changes in STK1 level and clinical reactions; i.e., STK1 was still high (>2 pM) or increasing in patients with SD or PD, but was low (<2 pM) or decreasing in patients with CR or PR (Table 5).

In another group of patients with lung, esophagus, gastric, thyroid, and head and neck carcinomas ($n = 223$), the STK1 values after tumor treatment were also related to the clinical reaction. In this group of patients, however, only the STK1 values after treatment were used because no STK1 values were available for these patients before treatment. Thus, the comparison between STK1 values and clinical reactions is limited because a single STK1 value may be not sufficient to judge the effect of treatment. STK1 values before and after start of the treatment showing decreasing or increasing STK1 values are a more reliable judgment, i.e., using internal controls. Furthermore, the

Table 4 STK1 values after treatment of patients with and without metastatic disease

Carcinomas	No metastasis	Metastasis
Lung	1.2 ± 1.4 ($n = 27$)	0.9 ± 0.9 ($n = 8$)
Esophagus	0.9 ± 1.0 ($n = 53$)	3.9 ± 7.2 ($n = 18$)
Gastric	1.0 ± 1.5 ($n = 13$)	2.0 ± 2.2 ($n = 4$)

Data are mean value ± SD. Values in parentheses are number of patients

Table 5 Number of patients with low/decreasing or high/increasing STK1 values by time after start of treatment in relationship to clinical reactions (CR, PR, SP, PD) of 40 carcinoma cases (lung, $n = 12$; esophagus, $n = 23$; gastric, $n = 5$)

Clinical reaction	STK1 low or decreased	STK1 high or increased
CR	10/11	1/11
PR	2/3	1/3
SD	6/21	15/21
PD	2/5	3/5

Low STK1 value, <2 pM; high STK1 value, >2 pM

follow-up time was only 2 months after the start of treatment. The results are summarized in Table 6. There was a tendency for CR patients to have lower STK1 values compared to SD or PD patients.

The predictive tool of STK1 for the outcome of therapy is more convincing when comparing head and neck and esophagus patients who received surgery and/or chemotherapy (Table 7). As already shown (see Fig. 3c), the STK1 values of the head and neck patients decreased significantly after surgery but not after chemotherapy. These results were correlated to a significantly higher number of CR patients undergoing surgery as compared to chemotherapy. Similar correlations between clinical reaction and STK1 value were found among patients with esophagus carcinoma. There was no significant difference in the number of CR patients ($P = 0.070$) and in the STK1 values ($P = 0.114$, see Fig. 3b) when the patients were treated with surgery or chemotherapy. Some few patients ($n = 4$) received surgery + chemotherapy. Two patients showed SD, and one each PD and CR. Mean STK1 value was 0.9 ± 0.8 pM. Stage I–III lung carcinoma patients treated with surgery + chemotherapy showed a higher number of CR and PR cases as compared to stage IV patients who only received chemotherapy (Table 7), which may be expected. However, the STK1 values decreased significantly more in the stage IV group of patients as compared to the stage I–III patients (see Fig. 3a). There were a few stage IV patients who received surgery only ($n = 3$) or surgery + chemotherapy ($n = 3$). The mean STK1 values were 4.4 ± 5.2 and 1.0 ± 0.8 pM, respectively, with clinical reactions corresponding to SD/PD and SD, respectively. Although there were only a few such patients, there may be a correlation between clinical reaction and STK1 value; i.e., higher STK1 values coincide with SD/SP. That 70% of the patients receiving chemotherapy had metastasis, whereas no metastasis was found among the patients who received surgery + chemotherapy, may be also influence the results.

Table 6 STK1 values of patients with different types of carcinomas in relationship to clinical reactions (CR, PR, SD, PD)

Carcinomas	CR	PR	SD	PD
Lung ($n = 21$)	0.9 ± 0.6	0.9 ± 0.6	1.2 ± 0.7	2.4 ± 6.2
Esophagus ($n = 81$)	0.9 ± 1.3	1.4 ± 1.3	0.8 ± 0.5	3.3 ± 6.6
Gastric ($n = 13$)	1.1 ± 1.7	–	–	2.9 ± 3.3
Head and neck ($n = 24$)	1.5 ± 1.1	1.2 ± 1.3	–	6.4
Thyroid ($n = 45$)	1.3 ± 1.0	1.2 ± 0.9	2.8	0.7 ± 0.1

Clinical reactions were judged after 1–2 months after collecting sera for STK1 analysis. Data are mean ± SD

Discussion

In this study, we chose three of the most frequent types of cancer in China, i.e., gastric, lung, and esophagus cancer. The cancer incidence rates (number of persons/100,000) for these types of malignancies are 40.8, 34.7, and 33.5, respectively; the corresponding mortality rates are 25.2%, 17.5%, and 17.4%. We also chose thyroid cancer, which has a general low cancer incidence rate in China overall (2.2) but is rather frequent in the Fujian Province where this study was performed. No information on the mortality rate of thyroid cancer is available. Head and neck cancer is a rare type in China; currently, no cancer and mortality incidence rates are available. The total cancer incidence rate in China today is about 200/100,000 persons. These types of tumors were also chosen depending on different growth rates and ability to cure; thyroid carcinoma represents a slow-growing tumor that is relatively easy to treat, whereas the prognosis for lung carcinoma is worse.

Table 7 Number of patients showing different types of clinical reactions after surgery and/or chemotherapy (chemo)

Therapy	CR	PR	SD	PD	*P* value (CR → PR, SD, PD)	Notes
Lung						
Surgery + chemo ($n = 13$)	7	3	3	0		Stage I–III patients
Chemo ($n = 8$)	0	4	2	2	0.011	Stage IV patients
Esophagus						
Surgery ($n = 61$)	32	3	9	16		
Chemo ($n = 20$)	6	6	5	3	0.070	
Head and neck						
Surgery ($n = 16$)	11	5	–	0		
Chemo ($n = 8$)	2	5	–	1	0.043	

The *P* values were calculated by chi-square test

Here we extended the clinical study to 1,247 patients of different types of malignancies to determine possible correlations between STK1 and clinical stages and clinical reactions and to confirm earlier results showing that STK1 can be used for monitoring tumor treatment in routine clinical settings. We found that STK1 correlated to clinical stages and clinical reactions and monitored the outcome of treatments. On the other hand there was no correlation of STK1 with pathological types (AC, SCC), in agreement with earlier studies on STK1 [24]. The complex relationship between STK1 and tumor size indicates that the serum level of TK1 is not related only to tumor volume. Because TK1 is closely related to growth [10–13], a different level of STK1 may also reflect the growth rate of the tumors in the patients. Thus, STK1 in combination with the tumor size may be able to identify patients with slow- or fast-growing tumors of the same tumor size. The clinical stages of the various subgroups of STK1/tumor size speak in favor of that idea, at least in some carcinoma patients. It seems that in patients with lung, thyroid, and gastric carcinomas, higher STK1 values with the same tumor size indicate a later clinical stage. However, in esophagus carcinoma patients, no clear correlations between STK1 value, tumor size, and stages were found. Furthermore, in lung carcinoma patients, small tumors and higher STK1 values seem to indicate an earlier clinical stage. Thus, although subgroups of various STK1/tumor size seem to correlates with clinical stage in some types of carcinoma, more investigations are needed to confirm this.

In patients after treatment, STK1 correlated well to the outcome of patients who were followed individually after start of the therapy but also in groups of patients before and after treatment independent of each other. We also found, in esophagus and gastric carcinoma patients with metastasis, that the STK1 values were not reduced after treatment as much as in patients without metastasis. We found that STK1 value could distinguish between the outcome of surgery and/or chemotherapy in patients with head and neck and esophagus carcinomas but not in patients with lung carcinomas.

The lack of CR cases among the lung cancer patients with stage IV receiving chemotherapy only, although showing significantly reduced STK1 values after surgery, could be explained by advanced cancer (stage IV) with metastasis. The physiological conditions of these patients when treatment was started were probably much worse than for those who had less-advanced tumors. The possibility to cure metastatic patients is also limited, which explains the lack of CR patients. The 5-year survival of stage IV patients is less than 2%. The corresponding survival time for stage I patients is less than 50%, for stage II patients less than 25%, and for stage III patients less than 10% [23]. It is still possible that STK1 values reflect chemotherapy-reduced tumor burden although the patient's general physiological condition is still poor. In this respect, it is important to note that TK1 is a proliferation marker related to proliferating tumor cells and thus reflects tumor burden rather than the general health condition of the patients.

The results in the present study are in accordance with recent studies on serological TK1. For example, in a study on low-risk breast patients with relapse, the STK1 level was significantly higher at 3 months after operation/adjuvant therapy as compared to nonrelapsed patients, and remained high during at least a further 33 months [20]. Similar results were found in a recent study on 400 breast cancer patients after surgery. Nine months before relapse was visible by imaging techniques, TK activity in serum was already elevated and continued to increase until relapse became visible [26]. In another study on 234 patients of different types of carcinomas, TK1 concentrations in serum correlated well with the outcome of the tumor therapy [24]. Earlier studies on limited numbers of carcinoma patients also supported the use of TK1 in serum for monitoring the results of cancer therapy (breast [27], gastric [28], lung [29], and bladder [22] carcinomas). Thus, the present study and earlier studies on STK1, together based on more than 1,700 cancer patients with different types of carcinomas, confirm that TK1 in serum is not only useful for evaluation of tumor progression but is also a useful tool for evaluation of the effect of tumor therapy.

The mean STK1 value of the healthy persons in this study was 0.4 ± 0.3 pM, which is in agreement with earlier results (see Xu et al. [24], $n = 761$, STK1 0.6 ± 0.4 pM; and HengZhi et al. [30], $n = 11{,}880$, STK1 0.3 ± 0.3 pM) confirming the low STK values found in this study.

Changes in STK1 values after surgery are predictable. Resection of the tumor of patients with no metastasis results in decreasing STK1 values while the presence of metastasis keeps the STK1 level unchanged. In the case of chemotherapy, changes of STK1 value may behave in different ways depending on the type of drug used, as has been studied in patients receiving systemic multidrug therapy, measuring TK activity [19, 31, 32]. The changes in TK1 activity found in these studies are also representative for changes in STK1 values. As already mentioned, TK1 is mainly present in S-phase cells. Therefore, drugs that interfere with DNA synthesis (S-phase) leading to cell disintegration should cause a peak of STK1 within a few days after start of the treatment. The magnitude of the STK1 peak may be reflecting the number of dead cells and thus the efficacy of the therapy. In such a situation, STK1 values before treatment and 3–4 weeks after treatment should be compared. On the other hand, a drug that affects cells in early phases of the cell cycle, when the

concentration of TK1 is low, or inhibits cell growth without disrupting the cells, may not show induction peaks of STK1. Instead, STK1 will be found continuously decreasing, as seen after surgery, if no metastases are present. The effect of intermittent chemotherapy should be evaluated by determination the STK1 values before, during, and after each treatment cycle.

The elevated levels of STK1 in patients with recurrence after curative surgery/adjuvant therapy give new options to evaluate the therapy in a short time period. It also provides a possibility to pay special attention to patients with risk for recurrence during early postoperative surveillance. STK1 also may enable improving the treatment and can probably precede imaging techniques, resulting in early pretreatment, which enables an increased chance of survival.

We conclude that STK1 correlates with clinical stage and clinical reaction and thus is useful for monitoring the outcome of tumor therapy, in particular, to distinguish between different types of treatments or to judge further effects of additional treatments. It provides decision support, allowing individual therapy for different carcinomas, avoiding overtreatment and/or changes of treatment strategy. The STK1 assay may enable increased survival and thus enhance the quality of life of cancer patients.

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Conflict of interest statement None.

Appendix

See Table 8.

Table 8 Number of patients in the various groups analyzed

Items	Lung	Esophagus	Gastric	Head and neck	Thyroid
Total number	303	363	69	138	374
Sex					
Man	212	269	52	109	67
Woman	91	94	17	29	307
Stage					
I	34	7	3	–	63
II	6	36	4	–	7
III	23	47	4	–	42
IV	24	39	8	–	13
Adenocarcinoma	14	4	10	–	–
Squamous	9	31	1	–	–
Low STK1/small	10	21	4	–	59
High STK1/small	3	7	9	–	7
Low STK1/large	26	46	0	–	39
High STK1/large	15	28	5	–	9
Treatment					
Before	238	267	37	115	327
After	65	96	32	23	47
Clinical response					
CR	7	38	–	13	–
PR	7	9	–	10	–
SP	5	14	–	0	–
PD	2	19	–	1	–
Metastais	8	18	4	0	0

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