A meta-analysis of serological thymidine kinase 1 as a marker for colorectal benign and malignant tumor risk assessment

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Abstract. The present study investigated whether a concentration of serum thymidine kinase 1 (STK1p) could be used to distinguish between healthy individuals, patients with colorectal benign tumors and individuals with colorectal cancer (CRC). The effectiveness of surgery on patients with CRC was monitored. A total of 20 publications containing patients with CRC (n=1,836), patients with colorectal benign tumors (n=774) and healthy controls (n=1,701) were analysed in the present meta-analysis. The publications were collected from PubMed, Embase, CENTRAL, CNKI, Wanfang, VIP and SinoMed databases from January 1, 2009 until August 31, 2019. Articles were analyzed according to sensitivity (Forest plot) and publication bias (Begg's plot, Egger's linear regression) using fixed or random effect models to calculate the weighted mean difference. Study quality was checked using the Newcastle-Ottawa Scale Document Quality Assessment Scale. The meta-analysis followed the PRISMA statement. The results revealed that STK1p significantly distinguished healthy individuals and those with colorectal benign tumors from patients with CRC, and from patients with benign tumors (P<0.000001). STK1p levels also decreased by 40% following surgery (P<0.0001), which corresponded to half-life of ~1 month. The quality of the present study was high and no bias was identified among publication. It was concluded that STK1p was a reliable biomarker for the early detection of benign lesions, which may therefore prevent their future development into colorectal malignancies. STK1p may also be used for the clinical dynamic monitoring of the effectiveness of surgery in patients with CRC. Combining STK1p with colorectal-associated biomarkers, in addition to the determination of tumor stage and grade may therefore be of use.

Introduction

According to the World Health Organization, the number of newly diagnosed colorectal cancer (CRC) cases will increase by 77% and the number of deaths by 80% by 2030 (1,2). The expected increase in CRC will mainly occur in less developed countries, due to the development of a lifestyle closed to Western countries (3-5).

The neoplastic transformation time of CRC is ~10-15 years (6-9), and the 5 year survival following treatment 50-60% (10,11). Reliable diagnostic, prognostic and predictive biomarkers are required (12). At present, the most accepted tests for CRC are fecal occult blood test, colonoscopy and sigmoidoscopy (13). Tumor node metastasis (TNM) staging and carcinoembryonic antigen level are also used. However, most of these tests have been reported to be too insensitive for accurate individual prognosis (14,15). Tumor biomarkers with a high sensitivity and specificity are required. In this meta-analysis, the use of serum thymidine kinase 1 (STK1p) concentration for prognosis and treatment monitoring in CRC patients is discussed.

STK1p has been proven useful for predicting recurrence and survival in many types of human cancer (16,17). TK1 is a kinase enzyme that converts deoxythymidine to deoxythymidine monophosphate and is involved in the synthesis of DNA, and thus related to cell growth rate (proliferation). The new-generation STK1p concentration assay shows an area under the curve (AUC) value of 0.96 (18), and is a more reliable assay than the serum TK activity (19,20) and serum TK1 sandwich ELISA (21) assays. Low STK1p values are associated with a better prognosis (16,18,22-27). The STK1p marker is an independent prognostic factor for survival in CRC patients (n=504) (28). STK1p can distinguish malignant patients from benign tumor patients and healthy individuals, as well as predict the prognosis of survival and relapse. STK1p can also monitor the effect of the treatment, and is a useful biomarker for predicting the development of malignancies and discovering early-stage tumors (18,26,28).

A number of studies have focused on the use of STK1p in colorectal benign and malignant tumor patients, but most of them included a limited number of cases, thus reducing the reliability of the conclusions. Therefore, 20 colorectal studies

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were collected for the present meta-analysis, in order to obtain a sufficient number of cases. The results showed that STK1p values are significantly higher in CRC patients, as compared to heathy individuals or patients with benign tumors. STK1p was also used to monitor the effect of the surgery.

Materials and methods

Literature search. A systematic literature search was conducted through the PubMed, Embase, CENTRAL, CNKI, Wanfang, VIP and SinoMed databases until August 31, 2019, using the following strategy keywords: ('thymidine kinase 1' or 'TK1') and ('colorectal' or 'colon' or 'rectal' or 'colorectum' or 'rectum') and ('cancer' or 'tumor' or 'carcinoma' or 'malignancy'). A more detailed description of the search strategy used is described in the beginning of the Results section. The literature search was restricted to human studies. There were no language restrictions.

The meta-analysis followed the PRISMA guidelines. The quality of the meta-analysis as a study was investigated using the Newcastle-Ottawa Scale Document Quality Assessment Scale (NOS). Only studies using the STK1p assay developed by us, tested in many clinical studies and statistically proved to be reliable were selected (summary in refs. 27,28), in order to guarantee the high quality of the STK1p results. Since no specific review protocol suitable for our meta-analysis was found, our own protocol was used.

All analyses were based on previously published studies, and therefore no ethical approval and patient consent were required.

Inclusion and exclusion criteria

Inclusion criteria. i) STK1p as an endpoint; ii) STK1p was measured by the kit developed by SSTK Ltd; iii) patients with adenomatous polyps were identified by clinical endoscopy and pathological diagnosis. Patients were identified by pathological diagnosis, classified as stage I-III and grade 1-3, and confirmed to have no residual tumor following surgery; iv) healthy individuals were used as the control group. Healthy people were defined as disease-free checked by different imaging, blood tests and other pathological methods.

Exclusion criteria. i) Insufficient data; ii) TK1 immunohistochemistry and TK1 activity; iii) Invalid research data, which included physiological stress responses such as immunological reaction, inflammation and activation of metabolic adenosine mediators. Oxidative stress was considered a surgical stress response, together with myocardial injury, sepsis, pulmonary edema, and kidney and liver failure, which could increase mortality; iv) Healthy people were excluded when containing diseases associated with tumors proliferation, such as precancerous (moderate/severe types of hyperplasia of breast, prostate, gastrointestinal, cervix, liver cirrhosis, refractory anemia). Also excluded were people with risk-diseases associated with tumors progression such as liver disease, moderate/severe fatty liver, high risk for hepatitis B, abnormal liver function, obesity and benign tumors (such as renal, thyroid); and any of the following conditions: severe cardiac disease; using any medication that could affect the STK1 levels such as exogenous hormone therapy; pregnancy; or acute illness such as inflammation/virus infection within 4 weeks.

Table I. No of publications in the group of controls, benign and malignant persons, no of samples and STK1p mean values in the various groups included in the present study.

Variables	Control	Benign	Malignant
Publications (n)	19	10	20
Samples (n)	1,701	774	1,836
STK1p (mean ± SD)	0.88±0.50	1.30±0.84	3.14±2.55
P-value	0.00078[a]	<0.0001[b]	<0.0001[c]

P-values corresponding to the comparison of STK1p values between groups. [a]Control vs. benigh group, [b]benigh vs. malignant group and [c]malignant vs. control group. SD, standard deviation; STK1p serum thymidine kinase 1.

Literature screening and data extraction

Primary screening. The title and abstract of literatures were carefully reviewed, and 10% of the excluded papers were randomly selected to check the concordance rate.

Secondary screening. After checking the abstracts, the full text of the papers was re-evaluated, and it was decided whether these papers should be included to the study or not, according to the criteria. Authors 1, 2 and 3 screened papers independently and discussed to reach an agreement; when met with a disagreement, the papers were rechecked by authors 5 and 6.

The following data were extracted from each study: First author's name, publication year, title of publication, published journal, study population, number of samples, design type and results.

Statistical analysis. RevMan 5.1 statistical software provided by Networks of Cochrane Review Groups and Stata 12.0 data analysis and statistical software were used (29). A heterogeneity test was performed at the beginning, and depending on the results, a fixed or random effects model was used. A fixed effects model with an I^2 of <50% or random effects model with an I^2 of >50% was used to calculate the weighted mean difference and 95% confidence interval. In addition, Funnel plot and Egger's linear regression test was used to assess literature bias. For the comparison of STK1p concentration among the different groups of controls and patients, one-way analysis of variance followed by a post hoc least significant difference test was performed. SPSS version 19 was utilized for statistical analysis (IBM Corp.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Literature search and study characteristics. The healthy control group included individuals with no evidence of tumors. Data from patients with colorectal neoplastic (adenomatous) polyps were used in the meta-analysis. Adenomatous polyps are benign tumors that originate from the mucus-secreting colonic epithelial cells (9). Tumors from CRC patients pathologically identified as clinical I-III degree and grade G1-G3 were defined as malignant. We had no information on whether
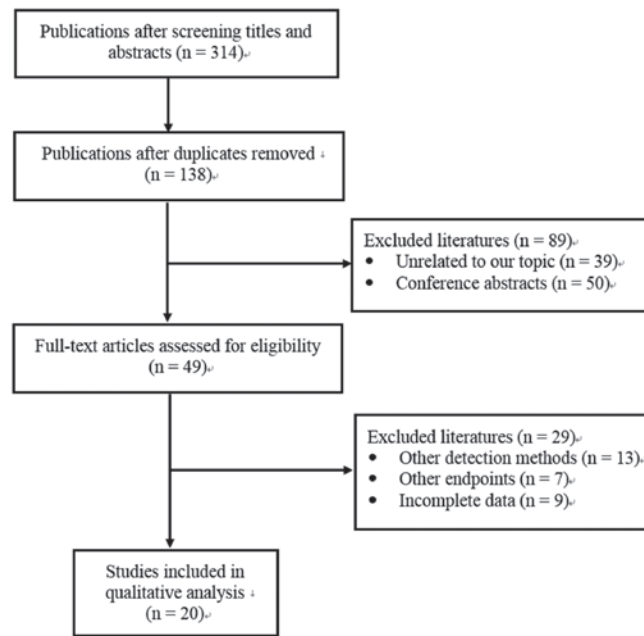
Figure 1. Flow diagram of the literature search and study selection process.

other types of tumors besides CRC were identified in the benign or malignant tumor groups.

As shown in Fig. 1, a total of 314 publications were initially identified through a search of the Pubmed, Embase, CENTRAL, CNKI, Wanfang, VIP and SinoMed databases. A total 176 publications were removed due to duplications and 138 were kept. Next, 89 publications were excluded from the remaining articles following a screening of the titles and abstracts, due to being conference abstracts or focusing on unrelated topics. Therefore, 49 full-text articles were assessed for eligibility. Among them, 13 were excluded, since another STK1 method was used instead of the SSTK dot blot ECL assay, 7 because they used different endpoints, and 9 due to incomplete data. Finally, 20 studies were included in this meta-analysis (30-49).

The number of manuscripts, patients involved and the STK1p mean values in the control, benign and malignant tumor groups are shown in Table I. Detailed information on the number of cases, age distribution, type of CRC, clinical stages grades and just before start of the surgery and one month after surgery reported in the individual publications are presented in Table II.

A total of 1,836 CRC patients were included in the 20 publications, including 774 benign tumor patients and 1,701 healthy individuals. The majority of CRC patients were male with an age-distribution of 16-85 years and colon cancer clinical stage of III and grade low/medium/high. The expression of STK1p increased significantly in the following manner: Controls < benign < malign (P<0.001). The quality of the meta-analysis as a study was checked by the NOS and was found to be high, with 19 publications meeting 8 of the requirements of NOS, and 1 publication meeting 6. The results are presented in Table III.

STK1p values in different populations. The STK1p values were significantly different between healthy individuals and benign tumor patients, healthy individuals and CRC patients, and benign tumor and CRC patients (Table I, Fig. 2A). The

number of publications involved among the healthy controls were 19, the benign 10 and the malignant 20, corresponding to 1,701, 774 and 1,836 persons, respectively. The STK1p values decreased significantly (40%) 1 month after surgery (Fig. 2B).

Meta-analysis statistical calculation. Significant values were calculated using statistical programs for meta-analysis (see Material and methods sections). The results for the different test groups are shown in Figs. 3 and 4. The statistical values were calculated between healthy individuals and benign tumor patients (Fig. 3B), healthy individuals and CRC malignant tumor patients (Fig. 3A), benign and malignant tumor patients (Fig. 3C), and before and after surgery (Fig. 3D).

STK1p of healthy individuals compared to benign tumor patients. In this statistical calculation 1,052 healthy individuals and 671 benign tumor patients from 9 studies were used. Based on a random effects model, the heterogeneity test showed that the STK1p value in healthy individuals was significantly lower than that in benign tumor patients (Fig. 3B).

STK1p of healthy individuals compared to CRC patients. Of the 20 publications received, 19 were used in this comparison, including 1,701 healthy individuals and 1,703 patients with CRC (Fig. 3A). A heterogeneity test based on the random effects model showed that the STK1p value in CRC patients was statistically higher than that in healthy individuals.

STK1p in benign tumor patients compared to CRC patients. Out of the 20 publications, 10 included data regarding STK1p in benign tumors and CRC (Fig. 3C). There was a total of 774 cases in the benign tumor group and 904 in the CRC group. A heterogeneity test based on the random effects model showed that the STK1p values were significantly higher in CRC patients, as compared to benign tumor patients.

Table II. Summary of the clinical data included in each selected publication.

Author/(Ref), year	Location	Control (n)	Benign (n)	CRC (n)	M (n)	F (n)	Age	Clinical stage (n)			Pathological grading (n)		CRC type		Surgery	
								I	II	III	Low diff.	Medium/ high diff.	Colon	Rectum	Before	After
An et al (36), 2016	Southwest	33		45	45		31-76			45			24	21	13	13
Huo et al (41), 2015	East China	32	35	76	51	25	36-83			76			76			
Li (46), 2012	Southwest	120	100	120	78	42	31-80			120			60	60	120	120
Li and Wang (49), 2009	East China	48	45	108	79	29	31-78			108				108		
Liu et al (40), 2015	South China	600	137	65	65		22-67			65			65			
Lu et al (44), 2014	South China	40	61	77	43	34	38-88			77			38	39		
Shen et al (47), 2011	East China	60	50	43	28	15	30-79			43			21	22	43	43
Tian et al (48), 2010	East China	33		66	45	21	25-72			66			66		66	66
Xia et al (39), 2015	East China	41		61	33	28	23-85			61	43	18	61		16	16
Qi et al (45), 2013	East China	45		104	104		32-81			104			52	52		
Zeng and Zhang (38), 2015	East China		103	133	77	56	32-84			133			133			
Zhang et al (42), 2015	Huazhong	40	36	150	88	62	30-78			150			150		150	150
Zhang et al (43), 2014	East China	161	162	64	64		35-84			64			35	29		
Zhu et al (35), 2017	Huazhong	52		82	49	33	36-68	17		65	34	48		82		
Zhu et al (37), 2015	East China	67	45	33	33		16-85	7	8	18				33	33	33
Fan et al (30), 2019	Northwest	60		50	23	27	60-70			50	31	19	50			
Jiang et al (32), 2018	East China	70		71	50	21	32-82	9	26	36	18	53	22	49		
Weng (31), 2018	South China	64		64	36	28	42-73	15	29	20				64		
Sun et al (33), 2018	Northeast	60		80	50	30	24-80			80	54	26	80			
Ning et al (34), 2018	South China	75		344	344			132		212			177	167		
Total number		1,701	774	1,836	1,385	451		180	63	1,593	180	164	1,110	726	441	441
Age distribution		16-85	35-84				23-85								30-85	30-85

CRC, colorectal cancer; M, male; F, female; diff., differentiation.

Table III. Literature quality evaluation by Newcastle-Ottawa Scale Document Quality Assessment Scale (NOS).

Author/(Ref), year	Is the definition adequate?	Representativeness of cases	Section selection of controls	Definition of controls	Comparability of cases and controls on the basis of design and analysis	Ascertainment of exposure	Exposure same method of ascertainment for cases and controls	Non-response rate
An et al (36), 2016	*	*	*	*	**	*	*	*
Huo et al (41), 2015	*	*	*	*	**	*	*	*
Li (46), 2012	*	*	*	*	**	*	*	*
Li and Wang (49), 2009	*	*	*	*	**	*	*	*
Liu et al (40), 2015	*	*	*	*	**	*	*	*
Lu et al (44), 2014	*	*	*	*	**	*	*	*
Shen et al (47), 2011	*	*	*	*	**	*	*	*
Tian et al (48), 2010	*	*	*	*	**	*	*	*
Xia et al (39), 2015	*	*	*	*	**	*	*	*
Zeng and Zhang (38), 2015	*	*			**	*	*	*
Zhang et al (42), 2015	*	*	*	*	**	*	*	*
Zhang et al (43), 2014	*	*	*	*	*	*	*	*
Zhu et al (35), 2017	*	*	*	*	**	*	*	*
Zhu et al (37), 2015	*	*	*	*	**	*	*	*
Qi et al (45), 2013	*	*	*	*	**	*	*	*
Fan et al (30), 2019	*	*	*	*	**	*	*	*
Jiang et al (32), 2018	*	*	*	*	**	*	*	*
Weng (31), 2018	*	*	*	*	**	*	*	*
Sun et al (33), 2018	*	*	*	*	**	*	*	*
Ning et al (34), 2018	*	*	*	*	**	*	*	*

* and ** indicates that the eight quality test options were fullfiled in the studies. See more explanations at http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp.

Table IV. Egger's linear regression test for the assessment of publication bias.

| Standard effect	Coefficient	Standard error	T value	p>|t|	95% CI
Control vs. benign					
Slope	-0.8601885	0.2631424	-3.27	0.014	-1.482421 to -0.2379556
Bias	4.134483	2.616981	1.58	0.158	-2.053694 to 10.32266
Control vs. malignant					
Slope	-2.533914	0.249529	-10.15	0.000	-3.060375 to -2.007454
Bias	1.303459	2.242794	0.58	0.569	-3.428422 to 6.03534
Benign vs. malignant					
Slope	-2.50849	0.6528756	-3.84	0.005	-4.014024 to -1.002956
Bias	5.555459	4.287575	1.30	0.231	-4.331706 to 15.44262
Before vs. after treatment					
Slope	1.414582	0.2186961	6.47	0.001	0.8524054 to 1.976758
Bias	-0.6030739	1.460007	-0.34	0.744	-4.256143 to 3.249995

CI, confidence interval.
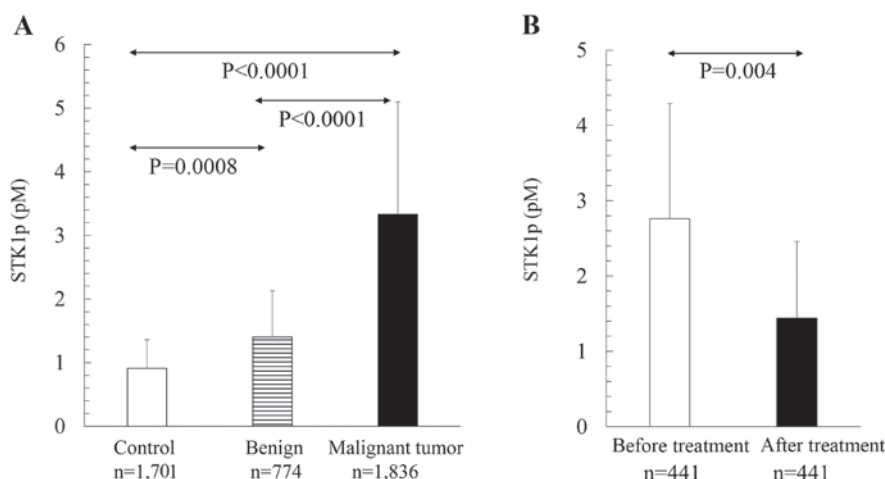
Figure 2. STK1p levels. (A) STK1p levels in healthy controls, benign patients and patients with malignant colorectal cancer patients. (B) STK1p levels in patients with malignant colorectal cancer before and after treatment. Data are presented as the mean ± standard deviation and significant values were determined using a t-test. STK1p, serum thymidine kinase 1.

STK1p level before and after surgery. All 20 publications included data regarding the STK1p level before and 1 month after surgery. A heterogeneity test, based on the random effects model, showed a significant decrease by 40% 1 month after surgery (Fig. 3D).

Sensitivity analysis. Sensitivity analysis was conducted to evaluate the effect of excluding any individual study. By excluding 1 article at a time in turn, the summary results of the remaining literatures did not substantially change (Fig. 4A).

Publication bias. Begg's Funnel-plot (Fig. 4B) and Egger' test (Table IV) were used to examine the potential publication bias. There was no evidence of bias between the healthy individuals and the benign tumor group, healthy individuals and the malignant group, the benign and malignant tumor groups, or the before and after treatment groups. The Egger's test showed that the possibility of potential bias from each comparison analysis

was very low (all P>0.05; Table IV). Based on these results, no significant publication bias was identified in this meta-analysis.

Discussion

Meta-analysis is a statistical analysis that combines the results of multiple studies (50-52). In the present study, a meta-analysis was performed according to the aforementioned inclusion and exclusion criteria. No bias was found. We also found that STK1p was able to distinguish between healthy individuals and benign tumor patients, as well as between healthy/benign tumor and malignant tumor patients. STK1p also monitored the results of the surgery. Thus, STK1p is a reliable biomarker for the prognosis of benign tumor and CRC patients, and a useful follow-up tool for surgery.

The STK1p was determined by an assay with a high sensitivity (0.80) and specificity (0.99), and an AUC value of 0.96. This assay is the most sensitive assay for TK1 in the serum on

A

Paper and years	Controls Mean	SD	Total	Malignants Mean	SD	Total	Weight	Mean difference IV, Random, 95% CI
An, et al., 2016	1.1	0.67	33	3.8	1.96	45	4.9%	-2.36[-3.25,-2.01]
Fan, et al., 2019	1.6	0.23	60	5.2	0.41	50	5.8%	-3.55[-3.68,-3.42]
Huo, et al., 2015	1.1	0.62	32	3	1.73	76	5.3%	-1.84[-2.28,-1.40]
Jiang, et al., 2018	0.4	0.1	70	4.6	1.8	71	5.3%	-4.20[-4.62,-3.78]
Li, et al., 2009	1.2	0.51	48	2.5	1.91	108	5.4%	-1.29[-1.68,-0.90]
Li, et al., 2012	1.1	0.98	120	2.5	1.87	120	5.4%	-1.40[-1.78,-1.02]
Liu, et al., 2015	0.8	0.2	600	2.5	1.23	65	5.6%	-1.69[-1.99,-1.39]
Lu, et al., 2014	0.8	0.26	40	2.9	3.17	77	4.6%	-2.10[-2.81,-1.39]
Ning, et al., 2018	0.7	0.48	75	3.4	3.87	344	5.3%	-2.70[-3.12,-2.28]
Qi, et al., 2013	1.1	0.57	45	5.5	3.31	104	4.8%	-4.36[-5.02,-3.70]
Shen, et al., 2011	0.6	0.5	60	2.4	3.05	43	4.1%	-1.87[-2.79,-0.95]
Sun, et al., 2018	1	0.14	60	3.5	0.36	80	5.8%	-2.52[-2.61,-2.43]
Tian, et al., 2010	0.9	0.12	33	3	0.38	66	5.8%	-1.95[-2.05,-1.85]
Weng, et al., 2018	1.1	0.32	64	3	1	64	5.6%	-2.04[-2.30,-1.78]
Xia, et al., 2015	0.8	0.87	41	3.5	2.33	61	4.8%	-2.40[-3.04,-1.76]
Zhang, et al., 2014	1.1	0.39	161	2.7	2.41	64	4.9%	-1.92[-2.51,-1.33]
Zhang, et al., 2015	1.1	0.56	40	2.4	1.79	150	5.5%	-1.29[-1.62,-0.96]
Zhu, et al., 2015	0.6	0.49	67	2.8	0.59	33	5.7%	-2.18[2.41,-1.95]
Zhu, et al., 2017	0.8	0.68	52	2.8	1.35	82	5.5%	-1.98[-2.33,-1.63]
Total (95% CI)			1,701			1,703	100%	-2.30[-2.65,-1.96]

Heterogeneity: Tau² = 0.52; Chi² = 652.23, df=18 (p<0.00001); I²=97%
Test for overall effect: Z = 13.28 (p<0.00001)

B

Paper and years	Controls Mean	SD	Total	Benigns Mean	SD	Total	Weight	Mean difference IV, Random, 95% CI
Fan, et al., 2019	1.64	0.23	60	2.31	0.37	45	12.6%	-0.67[-0.79,-0.55]
Huo, et al., 2015	1.13	0.62	32	1.39	0.91	35	9.0%	-0.26[-0.63,-0.11]
Li, et al., 2009	1.16	0.51	48	1.25	0.51	45	11.5%	-0.09[-0.30,-0.12]
Li, et al., 2012	1.09	0.98	120	1.19	1	100	10.7%	-0.10[-0.36,-0.16]
Liu, et al., 2015	0.8	0.2	600	1.35	0.82	137	12.4%	-0.55[-0.69,-0.41]
Lu, et al., 2014	0.83	0.26	40	1.58	0.85	61	11.2%	-0.75[-0.98,-0.52]
Shen, et al.,2011	0.55	0.5	60	1.35	0.82	50	10.7%	-0.80[-1.06,-0.54]
Zhang, et al.,2015	1.11	0.56	40	1.18	0.65	36	10.5%	-0.07[-0.34,-0.20]
Zhu, et al., 2017	0.8	0.68	52	0.92	0.77	162	11.3%	-0.12[-0.34,-0.10]
Total (95% CI)			1,052			671	100%	-0.39[-0.58,-0.19]

Heterogeneity: Tau² = 0.07; Chi² = 63.09, df=8 (p<0.00001); I²=87%
Test for overall effect: Z = 3.9 (p<0.0001)

C

Paper and years	Benigns Mean	SD	Total	Malignants Mean	SD	Total	Weight	Mean difference IV, Random, 95% CI
Fan, et al., 2019	2.31	0.37	45	5.19	0.41	50	10.5%	-2.88[-3.04,-2.72]
Huo, et al., 2015	1.39	0.91	35	2.97	1.73	76	10.0%	-1.58[-2.07,-1.09]
Li, et al., 2009	1.25	0.51	45	2.45	1.91	108	10.2%	-1.20[-1.59,-0.81]
Li, et al., 2012	1.19	1	100	2.49	1.87	120	10.2%	-1.30[-1.69,-0.91]
Liu, et al., 2015	1.35	0.82	137	2.49	1.23	65	10.3%	-1.14[-1.47,-0.81]
Lu, et al., 2014	1.58	0.85	61	2.42	3.17	77	9.3%	-1.35[-2.09,-0.61]
Shen, et al., 2011	1.35	0.82	50	1.93	3.05	43	8.6%	-1.07[-2.01,-0.13]
Zheng et al., 2015	1.34	0.63	103	1.93	0.96	133	10.5%	-0.59[-0.79,-0.39]
Zhang, et al., 2015	1.18	0.65	36	2.4	1.79	150	10.2%	-1.22[-1.58,-0.88]
Zhu, et al., 2017	0.8	0.68	162	2.78	1.35	82	11.3%	-1.86[-2.18,-0.78]
Total (95% CI)			774			904	100%	-1.43[-2.07,-0.78]

Heterogeneity: Tau² =1.03; Chi² = 362.74, df=9 (p<0.00001); I²=98%
Test for overall effect: Z = 4.32 (p<0.0001)

D

Paper and years	Before treat. Mean	SD	Total	After treat. Mean	SD	Total	Weight	Mean difference IV, Random, 95% CI
An, et al., 2016	3.07	1.54	13	1.24	1.04	13	7.5%	1.83[0.82,2.84]
Li, et al., 2012	2.49	1.87	120	1.61	0.97	120	17.4%	0.88[0.50,1.26]
Shen, et al., 2011	2.42	3.05	43	1.6	2.48	43	6.1%	0.82[0.35,1.99]
Tian, et al., 2010	2.97	0.38	66	1.53	0.41	66	21.3%	1.44[1.31,1.57]
Xia, et al., 2015	2.56	1.15	16	0.96	0.46	16	13.%	1.60[0.99,2.21]
Zhang, et al., 2015	2.4	1.79	150	1.63	1.08	150	18.2%	0.77[0.44,1.10]
Zhu, et al., 2015	3.41	0.92	33	1.52	0.79	33	16.6%	1.89[1.48,2.30]
Total (95% CI)			441			441	100%	1.31[0.97,1.65]

Heterogeneity: Tau² = 0.14; Chi² = 28.07, df=6 (p<0.0001); I²=79%
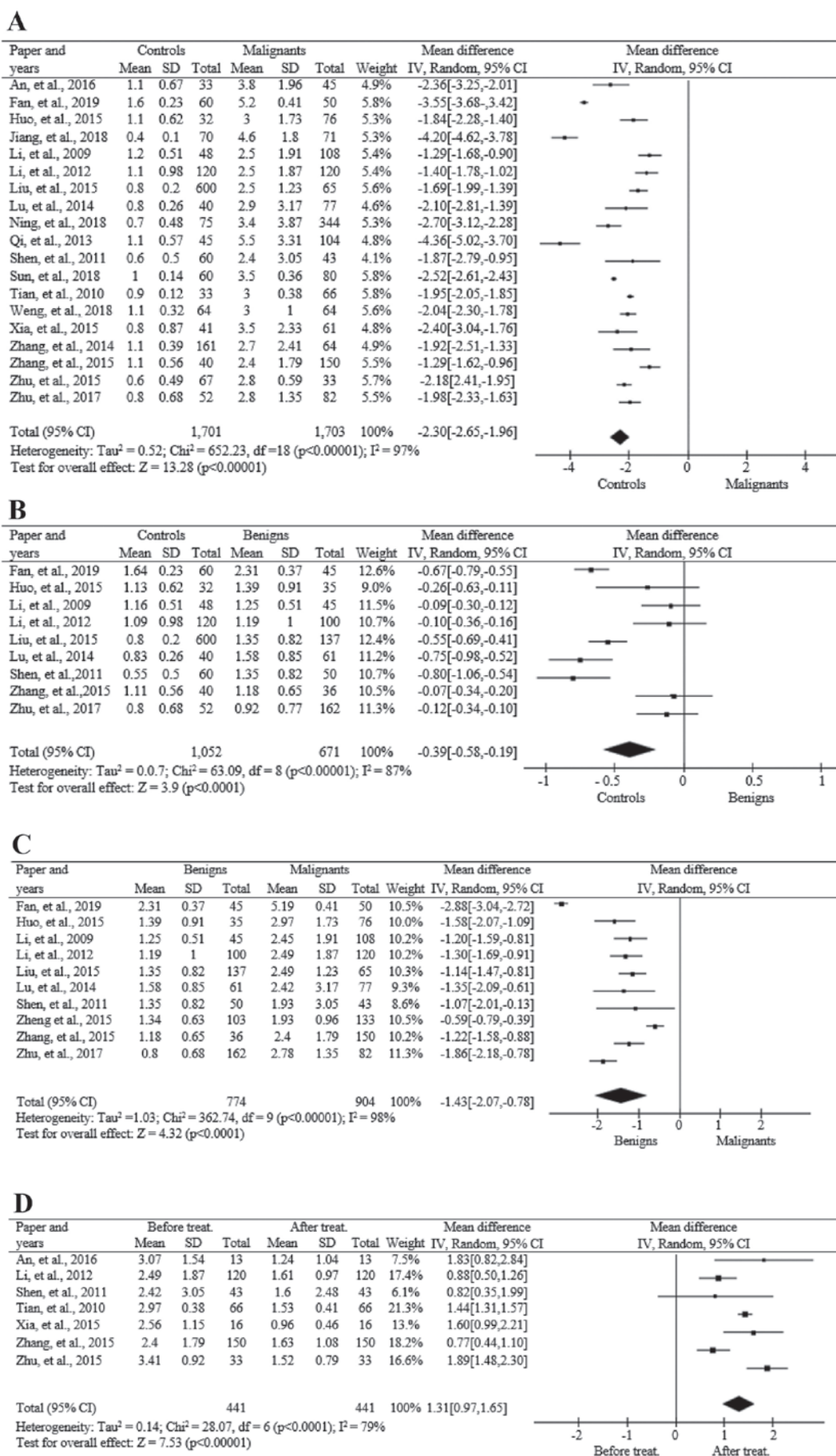Test for overall effect: Z = 7.53 (p<0.00001)

Figure 3. Forest plots for correlation analysis. Forest plots were assessed between (A) healthy controls vs. malignant patients, (B) healthy controls vs. CRC benign patients, (C) benign patients vs. CRC malignant patients and (D) before vs. after surgery. Black diamonds indicate the mean value. CRC, colorectal cancer; SD, standard deviation; CI, confidence interval; IV, inverse variation.
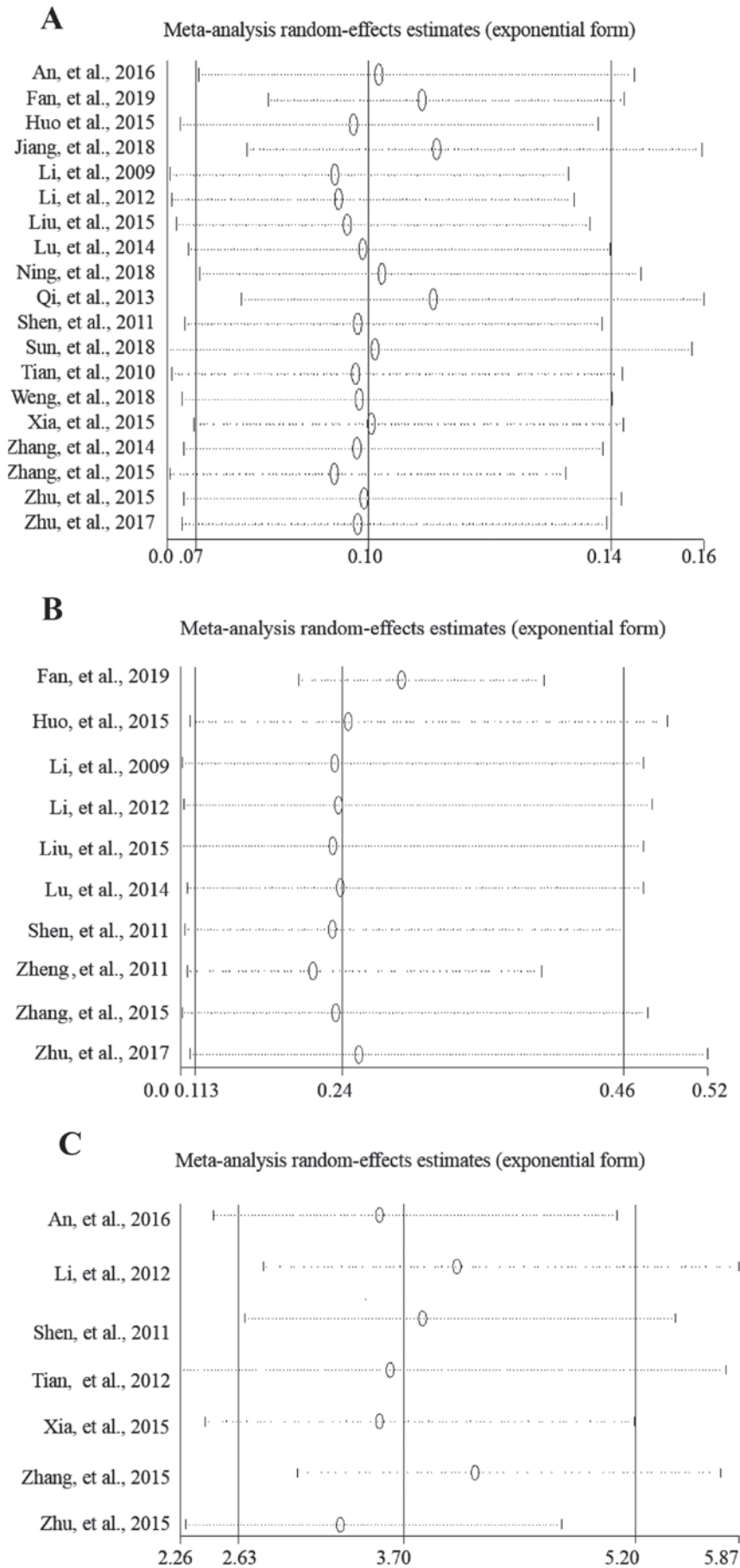
Figure 4. Study sensitivity analyses and the evaluation of mean differences. Evaluations were performed between (A) healthy control group and the colorectal malignancy group, (B) the benign disease and the colorectal malignancy group and (C) between the results obtained before and after surgery in the colorectal cancer group.

the market today (16). The mean value of STK1p in the benign tumor patients was 1.64-fold higher than that in healthy individuals, but significantly lower than that in CRC patients (1.60-fold). Although the mean STK1p value in benign tumor patients was higher than that in healthy individuals, there was a deviation, with both low and high individual STK1p values. The benign tumor patients with higher STK1p values (>2.0 pM) may be in a higher risk of developing malignancies than benign tumor patients with low STK1p values (<2.0 pM). Based on a health screening study (n=35,365) (18), where patients with a STK1p value of >2.0 pM were found to have a 3-5 times higher risk to develop a malignancy, we concluded that the risk for developing colorectal malignancies from a benign colorectal tumor should be higher in benign tumor patients with an STK1p value of >2.0 pM. On the other hand, benign tumor patients with STK1p values of <2.0 pM should have a low risk of developing CRC. STK1p was also found to have a prognostic potential in a randomised clinical trial that included patients with CRC (n=504) (28). Patients were followed up for 3-8 years. STK1p was compared with pathological stage and grade, lymph node metastasis, gender and age in relation to survival. A significantly worse survival was found among patients with high STK1p values (>0.9 pM), as compared to patients with low STK1p values (≤0.9 pM) (P<0.0001). Cox regression analysis demonstrated that STK1p, clinical stage and lymph node metastasis were independent prognostic factors, but Dukes' stage (P=0.633), sex (P=0.976) and age (P=0.520) were not.

In the present meta-analysis, the cut-off STK1p value was set to 0.9 pM, based on receiver operating characteristic statistical analysis. It is important to understand that the cut-off value of STK1p may differ depending on the type of tumor and how the measurement of STK1p was performed (healthy screening, clinical trials, etc.).

CRC is a heterogeneous disease, with most cases originating from polyp precursors. Different clinical stages and pathological grades of CRC can lead to heterogeneity.

CRC, a complex disease, is caused by both genetic and environmental factors. Certain studies have shown that inherited genetic factors account for ~35% of the disease etiology (53,54). It has been suggested that there may be two distinct categories of cancer: Right- and left-sided colon cancers that arise proximally or distally to the splenic flexure, respectively (55). A meta-analysis on vitamin E concentration in the serum suggested that serum vitamin E concentration was lower in patients with CRC than in healthy controls. Reduced serum vitamin E levels may therefore be a risk factor for CRC. However, prospective cohort studies are still required to assess the risk of serum vitamin E on CRC in the future (56). Although the original Dukes staging system has been modified several times, the extent of cancer invasion through the bowel wall and that of regional lymph node invasion is still the mainstay of TNM staging for CRC. A 17,641 patient-cohort study demonstrated that right- and left-sided colon carcinoma (CC) are significantly different in terms of epidemiological, clinical and histological parameters. Right-sided CC has been found to have a worse prognosis. These discrepancies may be due to genetic differences that determine distinct carcinogenesis and biological behaviour (57). There was no significant difference in recurrence rates between right- and left-sided CC and rectum carcinoma (RC), but the right-sided CC had a worse prognosis than left-sided CC and RC, possibly due to more advanced staging and fewer

curative resections (58). Another study demonstrated that the patients with stage I right-sided CC had a significantly better 5 year disease free survival rate than those with left-sided CC; however, no significant difference was observed in the distribution of the first patients with recurrence (59). A retrospective design and single-institution study showed that patients with right-sided colon cancers presented with a significantly increased risk of locoregional recurrence. Right-sided location, female sex, T4 disease, lymph node metastasis, and perineural invasion are independent risk factors for the locoregional recurrence of colon cancer (60). Qin *et al* (61) found that right- and left-sided colon cancer had significantly different clinicopathological characteristics. Right-sided colon cancer had a higher incidence of recurrence than left-sided colon cancer. Patients with stage III right-sided colon cancer had a worse prognosis than those with stage III left-sided colon cancer. In addition, CRC results may vary from region to region, resulting in heterogeneity, depending on patients' genetic characteristics and living conditions.

With regard to the heterogeneity in the STK1 results, in a preliminary CRC study on 492 patients (Prof. Desong Wan, Sun Yet-Sen University Cancer Centre, China) no significant difference (P≈0.628-0.645) was found in the STK1 values between right-(n=91) and left-(n=124) side colon cancer, and rectum (n=277). The STK1p value of right colon, left colon and rectum was 1.8±1.8, 1.9±1.8 and 1.9±1.8 pM, respectively. STK1p is therefore useful for assessing the proliferation rate in CRC serum samples.

Monitoring the response to treatment is important. Previous studies have shown that STK1p can be used not only to predict prognosis, relapse and survival, but also to monitor tumor treatment (28). In this meta-analysis, the half-life time of STK1p following surgery was found to be ~1 month, which is the half-life time identified following surgery in patients with lung cancer (26) and gastric carcinoma (62). In the case of gastric carcinoma, the STK1p values were significantly reduced to 52.7% 35 days after open surgery (P=0.0106). On the contrary, in the patients with distant metastases, the STK1p value increased to 173% at 35 days post-operatively. There was no significantly difference in TK activity. Similar results were also found in breast cancer (20). However, in the case of minimally invasive surgery, the half-life time of STK1p in patients with bladder carcinoma was only 1 week (63).

It is recommend that STK1p is combined with other tools to evaluate the treatment effect on patients with CRC. This will help individual treatment planning. The following parameters in combination with STK1p should be considered when designing a CRC study: i) CRC is a heterogeneous disease (63), the majority of which is developed from polyp precursors. Therefore, a complete study should use tools useful for the early detection, diagnosis, prognosis and management of CRC development from benign tumors; ii) Since CC and RC are two different types of malignancy (64), they should be evaluated separately; the same goes for right- and left-sided CC and RC; iii) While monitoring the effect of the treatment, the STK1p levels may change depending on clinical stage/grade and tumor type on an individual bases; iv) Since CRC results may differ between living area, depending on the genetic properties and living conditions of the patients, studies should include data from different health centres and oncology hospitals.

In summary, STK1p could potentially be used for the early detection of benign lesions to prevent their future development into colorectal malignancies, as well as for individual clinical dynamic monitoring of the results of surgery in patients with CRC. The combination of STK1p with colorectal imaging tools after treatment can provide a precise evaluation of the results of the therapy. Together with the use of colorectal-related biomarkers, tumor stage and grade for predicting the risk of relapse, STK1p can help doctors develope more accurate, individualized and rational treatment plans for patients.

Availability of data and materials

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Authors' contributions

LD, HM, AH and SX searched the literature, independently extracted the data, produced the figures and tables, and discussed the articles until an agreement was reached. JZ, EH and SS carefully re-analyzed all results. LD, HM and AH wrote the first version of the manuscript. EH and SS revised the introduction and discussion sections of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

JZ is the owner of SSTK Ltd., which produces the STK1p kit. Authors declare they have no competing interests.

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