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Prognostic analysis of Yes-associated protein 1 in patients with colorectal cancer. A systematic review and meta-analysis

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ABSTRACT

Background: colorectal cancer (CRC) is the most common carcinoma worldwide, but a lack of effective prognostic markers limits clinical diagnosis and treatment. Yes-associated protein 1 (YAP1) is an effector of the HIPPO-pathway, which plays a critical role in cancer development and prognosis, including CRC. However, previous

reports have suggested that it plays a dual role in CRC.

Methods: a meta-analysis using RevMan 5.4 and Stata 14.0 was performed to evaluate the relationship between YAP1 and clinical outcomes of CRC, after searching for eligible studies in the PubMed, Web of Science and Embase databases. Online datasets GEPIA and LOGpc were also used to calculate survival results and for comparison with the meta-analysis results. Besides, “DESeq” packages were used for the expression analysis of YAP1 from the TCGA dataset.

Results: YAP1 was overexpressed in the cancer tissues when compared to normal tissues in patients with CRC from the TCGA database ($p = 0.000164$) and GEPIA database. A total of 10 studies involving 2305 patients from the literature were selected. Pooled HR indicated that overexpression of YAP1 was associated with poor clinical outcomes (HR = 1.70, 95 % CI: 1.28-2.26, $p = 0.0003$). Subgroup analysis showed a clear correlation between overexpression of YAP1 and worse survival rate in Chinese patients (HR = 1.94, 95 % CI: 1.40-2.69, $p = 0.0001$), nuclear YAP1 overexpression (HR = 2.07, 95 % CI: 1.29-3.31, $p = 0.003$), 60 months of follow-up (HR = 1.89, 95 % CI: 1.30-2.73, $p = 0.0008$), IHC test (HR = 1.65, 95 % CI: 1.17-2.33, $p = 0.005$), IHC combined with other tests (HR = 1.77, 95 % CI: 1.13-2.77, $p = 0.01$) and multivariate analysis (HR = 1.70, 95 % CI: 1.24-2.31, $p = 0.0009$). Nevertheless, disease-free survival (DFS) showed no significant results in the patients with CRC in our meta-analysis (HR = 1.38, 95 % CI: 0.51-3.75, $p = 0.52$) as well as in the GEPIA and LOGpc databases. Meanwhile, YAP1 overexpression was also significantly associated with worse overall survival (OS) in GSE17536, GSE40967, GSE29623 and GSE71187.

Conclusion: YAP1 overexpression is common in CRC tissues. Overexpression of YAP1 in CRC patients, particularly in the nucleus, might be related to shorter OS, maybe in the early stages. YAP1 could serve as a potential predictor of poor prognosis in CRC.

Keywords: YAP1. Colorectal cancer. Prognosis. Meta-analysis. Online databases.

INTRODUCTION

Colorectal cancer (CRC) is widely diagnosed, especially in young patients (1), and is

currently the third most common cancer and second major cause of cancer-related death worldwide (2). The incidence and mortality of CRC differ between genders and geographical regions. CRC is the second most common cancer in women and third most common cancer in men. The highest incidence is observed in developed countries and is now increasing in developing countries; it is expected to reach 2.5 million new cases in 2035 (3,4). Currently, early resection and systemic therapy are very useful for CRC; however, metastasis and recurrence remain the main drivers of poor prognosis (3,5,6). Therefore, effective predictors of CRC need to be identified.

Yes-associated protein 1 (YAP1) was first detected in the fly (7); it is a downstream effector of the HIPPO-pathway in mammals. The HIPPO-pathway regulates cell growth and apoptosis by phosphorylating and inhibiting YAP1 (8,9). YAP1 translocates to the nucleus to function as a co-transcriptional co-activator for gene expression (9-11). Moreover, YAP1 can function as an oncogene in pancreatic cancer (12), hepatocellular carcinoma (13), bladder cancer (14), lung cancer (15), gastric cancer (16), and CRC (17). In contrast, YAP1 functions as a suppressor in breast cancer (18), CRC (19), and hematological cancer (20). In addition, YAP1 is clearly correlated with many clinico-pathological features, TNM stages, lymphatic metastasis, and distant metastasis (21,22). Most commonly, YAP1 acts as a poor prognostic factor for many cancers, including CRC (23-26), while it is also reported to be a protective factor for CRC prognosis (19,27). Thus, it is necessary to clarify the relationship between YAP1 and CRC, and to investigate the possible factors that lead to this paradox. Hence, a meta-analysis of relevant studies was conducted to evaluate the prognostic value of YAP1 in CRC. We also investigated the survival results in the GEPIA and LOGpc databases to identify potential influencing factors for this discrepancy.

METHODS

Search strategy

We searched the “PubMed”, “Web of Science” and “Embase” databases using combined “mesh” and “entry items” as keywords. The keyword combination used was as follows: “Colorectal Neoplasms(Mesh(OR Colorectal Neoplasm OR Neoplasm,

Colorectal OR Neoplasms, Colorectal OR Colorectal Tumors OR Colorectal Tumor OR Tumor, Colorectal OR Tumors, Colorectal OR Colorectal Cancer OR Cancer, Colorectal OR Cancers, Colorectal OR Colorectal Cancers OR Colorectal Carcinoma OR Carcinoma, Colorectal OR Carcinomas, Colorectal OR Colorectal Carcinomas” and “YAP1 OR YAP1 (Yes-associated) protein, human (Supplementary Concept)” and “Prognosis(Mesh(OR Prognoses OR Prognostic Factors OR Factor, Prognostic OR Factors, Prognostic OR Prognostic Factor OR survival OR outcome”. Also, the references of the eligible papers were reviewed to identify additional studies. The detailed search strategy is shown in supplementary files. The meta-analysis followed the PRISMA statement. We also contacted the authors for comprehensive information, if necessary.

Inclusion and exclusion criteria

An eligible study met the following inclusion criteria: 1) studies investigated the relationship between YAP1 expression and prognosis of CRC patients; 2) YAP1 expression was measured by polymerase chain reaction (PCR) or immunohistochemistry; 3) studies contained the relevant information of patients; and 4) studies provided HRs or Kaplan-Meier survival curves.

Studies were excluded based on the following criteria: 1) studies were meta-analyses, letters, abstracts, reviews or case reports; 2) studies did not have complete data or the data were questionable or the data came from public databases; 3) studies were not conducted on humans; and 4) studies included patients who were treated with drugs or radio-chemical therapeutics.

Data extraction

The included studies were independently reviewed by two researchers. The following information were extracted from the included studies: the name of the first author, the year of publication, country, number of cases and age, YAP1 location (cytoplasm or nucleus), cases of YAP1 overexpression, HRs and 95 % confidence intervals (CI). If the study reported the HR and 95 % CI, we extracted them directly; if not, we extracted the data from Kaplan-Meier survival curves using Engauge Digitizer

10.8 (28). If the study included both multivariate and univariate results, we selected the multivariate results for our meta-analysis. If the studies incorporated both cytoplasmic or nuclear analysis results, we chose both for our meta-analysis.

Quality assessment

The quality assessment of eligible studies was performed with the Newcastle Ottawa Quality Assessment Scale (NOS) by two researchers, and the studies with a score ≥ 6 were considered as high quality.

YAP1 expression analysis and prognostic analysis in online datasets

To further investigate the role of YAP1 in the prognosis of patients with CRC, we also performed the survival analysis with the online datasets GEPIA and LOGpc, as well as the expression analysis in TCGA dataset using “DESeq” packages.

Statistical analysis

We used the RevMan software 5.4 to perform the meta-analysis. A p -value < 0.05 was considered to be statistically significant, unless otherwise specified. OS, DFS and DSS were selected together for the outcome, pooled HR and 95 % CI were calculated using a random-effects model to determine the correlation between YAP1 expression and survival outcome, and the pooled HR was examined using the z-test. A pooled HR > 1 indicated a positive correlation, while < 1 indicated a negative correlation. Heterogeneity was measured by the I^2 statistic test and chi-square test. $I^2 > 50\%$ or $p < 0.05$ indicated heterogeneity, so we chose a random-effects model for the analysis; otherwise we selected a fixed-effects model. Subgroup analysis incorporated YAP1 subcellular location, follow-up time, analysis types, method of YAP1 testing, and ethnicity. Sensitivity analysis using Stata 14.0 was conducted by excluding one study at a time to evaluate the influence of a single study on all the studies. Furthermore, publication bias was assessed by a Funnel plot and Egger's test.

RESULTS

Study selection

We chose 225 studies from the three databases “PubMed”, “Web of Science” and “Embase”, after scanning the title and abstract, of which 208 were excluded because they were not related to YAP expression, did not study human CRC, were duplicates, laboratory studies, studies related to drugs, conference abstracts, letters, reviews or meta-analyses. Ten studies were excluded due to the following reasons: 1) without complete or useful data; 2) drug studies; 3) data from databases; and 4) not about CRC. Finally, seven articles were eligible for our meta-analysis (21,23-25,29-31). Additionally, three papers meeting the inclusion criteria from the selected studies were also enrolled for our meta-analysis (26,27,32). The flow chart of the study selection process is shown in figure 1.

Study characteristics

Among the eligible studies between 2013 and 2020, eight were from China and two were from Korea, involving 2305 patients. YAP1 was mainly expressed in the cytoplasm in one study, in the nucleus in three studies, and in both cytoplasm and nucleus in two studies, while the other studies did not describe the location. YAP1 expression was detected by IHC in eight studies and by IHC + q-PCR, IHC-q-PCR + WB in two studies. The exact follow-up duration was mentioned in five studies, and the follow-up duration was about 60 months in the majority of studies, while in two studies it was over 100 months. HRs and 95 % CI were directly retrieved from eight articles and calculated from Kaplan-Meier survival curves in two articles. The mean NOS score of the selected articles was 7.6, with a minimum of 7 and a maximum of 9. The principal characteristics of the included studies are presented in table S1.

Publication bias and sensitivity analyses

Publication bias was evaluated by a funnel plot (Fig. 2), as well as Egger’s test as conducted by the Stata software ($p = 0.164$) (Fig. S1); the results showed no obvious bias in the enrolled articles. Moreover, a sensitivity analysis was conducted using the random-effects model by sequential omission, and the result was not influenced by any single study (Fig. 3).

Meta-analysis results

Overall analysis

We evaluated the prognostic significance of YAP1 overexpression on overall survival (OS), disease-free survival (DFS) and disease-specific survival (DSS) of CRC patients. Nine studies involving 1386 patients were included for the meta-analysis of OS (details in table S1); heterogeneity was found among the studies ($I^2 = 53\%$, $p = 0.02$), hence a random-effects model was chosen and the result showed that YAP1 overexpression regardless of subcellular location was associated with poor OS (HR = 1.70, 95 % CI: 1.28-2.26, $p = 0.0003$) (Fig. 4). Two studies were included to evaluate DFS, and the result of combined HR with a random-effects model demonstrated no obvious association between YAP1 overexpression and DFS in CRC patients (HR = 1.38, 95 % CI: 0.51-3.75, $p = 0.52$) (Fig. S2).

Subgroup analysis

Due to the heterogeneity present, we conducted a subgroup analysis according to five factors (YAP1 subcellular location, follow-up time, analysis type, method of YAP1 testing and ethnicity). As shown in table 1, the subgroup analysis by ethnicity suggested that overexpression of YAP1 was associated with poor survival in Chinese patients (HR = 1.94, 95 % CI: 1.40-2.69, $p = 0.0001$) but not in Korean patients (HR = 1.23, 95 % CI: 0.83-1.82, $p = 0.31$). The subgroup analysis of YAP1 subcellular location indicated that YAP1 was mainly expressed in the nucleus (HR = 2.07, 95 % CI: 1.29-3.31, $p = 0.003$) and expression in the cytoplasm was not statistically significant (HR = 0.90, 95 % CI: 0.49-1.68, $p = 0.75$). A stratified test showed that IHC alone and IHC combined with other methods were effective for survival analysis (HR = 1.65, 95 % CI: 1.17-2.33, $p = 0.005$; HR = 1.77, 95 % CI: 1.13-2.77, $p = 0.01$). In the subgroup analysis, although the multivariate analysis showed significance with poor prognosis (HR = 1.70, 95 % CI: 1.24-2.31, $p = 0.0009$), the univariate analysis did not show this (HR = 1.61, 95 % CI: 0.60-4.31, $p = 0.34$). When grouped according to different follow-up durations, the shorter subgroup indicated that YAP1 overexpression was a predictor of poor OS (HR = 1.89, 95 % CI: 1.30-2.73,

$p = 0.0008$), while in the longer duration subgroup this was not the case ($HR = 1.92$, 95 % CI: 0.88-4.20, $p = 0.1$).

YAP1 expression and prognostic analysis in online databases

R 4.1.0 was used for the differential expression analysis between the tissue of patients with CRC and normal tissues. There were in total 602 cases containing 48 normal tissues and 554 cancers. Using DESeq packages, it is clear that YAP1 is overexpressed in cancers ($p = 0.000164$), as well as in GEPIA (Fig. S3). Next, we analyzed the association between YAP1 and CRC prognosis. The results showed that there was statistical significance between YAP1 and OS in GSE17536, GSE40967, GSE29623 and GSE71187 (Fig. S4), but not between YAP1 and DFS in LOGpc (Table S2).

DISCUSSION

Colorectal cancer (CRC) is a major public health problem worldwide, which increases the economic burden of disease. Although several predictors have been identified (33,34), their application is clinically limited, especially in the early stage. Overexpression of YAP1 is widely reported in cancer tissues compared to normal tissues, and YAP1 is implicated in the carcinogenesis of cancers. Elevated expression of YAP1 was reported to promote tumorigenesis and progression of CRC, while knockdown of YAP1 was found to repress CRC progression and growth (35). Xu et al. reported that YAP1 levels combined with plasma CEA levels are prognostic biomarkers in the early stages of CRC (21). In our meta-analysis, the pooled result showed that higher YAP1 expression was related to poor OS of CRC patients compared to the lower expression group, which is consistent with the results in GSE17536 and GSE40967. However, no significant relationship was found between YAP1 and DFS both in our meta-analysis and online databases. A subgroup analysis was performed by ethnicity, YAP1 testing methods, YAP1 subcellular location, HR analysis type, and follow-up time. The results indicated that Korean patients, univariate analysis, and follow-up duration over 100 months showed no clear association between YAP1 overexpression and OS.

YAP1 is expressed in the cytoplasm and the nucleus (27,36). YAP1 shuttles between the cytoplasm and the nucleus when the HIPPO-pathway is dysregulated, and different subcellular locations may produce different biological effects. Our meta-analysis indicated a strong positive correlation between overexpression of YAP1 in the nucleus and poor survival rate, while YAP1 in the cytoplasm showed no statistical significance. These results suggested that nuclear YAP1 could be an effective predictor of CRC, and the previously reported dual roles of YAP1 in CRC may be caused by YAP1 subcellular location in different situations. Further studies are needed to clarify this issue.

However, there was discrepancy between our meta-analysis results and GEPIA results. In our meta-analysis, overexpression of YAP1 indicated a poor OS, while there was no statistically significant association in GEPIA. We found several cross points from the K-W curves ranging from about 60 months to 100 months, and we conducted a subgroup analysis according to length of follow-up. Intriguingly, a follow-up time about 60 months showed a significant statistic difference; however, follow-up time over 100 months showed no meaningful association, which led us to consider whether YAP1 is an early prognosis factor — further investigation is needed going forward. Zhang reported that loss of YAP1 indicated poor prognosis in terms of OS in CRC during long-term follow-up (27). Xu et al. also reported that YAP1 and CEA are prognostic biomarkers for early stage patients with CRC (21). So we speculated that follow-up duration may be responsible for the previously reported dual roles of YAP1 in CRC. Meanwhile, different cutoff values led to different results, even completely opposite outcomes in databases. However, the role of YAP1 overexpression in predicting the prognosis of CRC patients was confirmed in our meta-analysis. Further studies based on follow-up duration and cutoff values are needed.

This meta-analysis had certain limitations such as heterogeneity among the included studies, cutoff values, small sample sizes, short follow-up durations, lack of effective data in some studies, and inclusion of Asian patients only.

CONCLUSION

YAP1 overexpression is common in CRC tissues, and such overexpression (particularly in the nucleus) is associated with poor survival, maybe mainly in the early stages. Further, well-designed (especially focusing on follow-up time and cutoff values), prospective, multicenter, large-sample studies are needed to confirm the clinical value of YAP1 in CRC.

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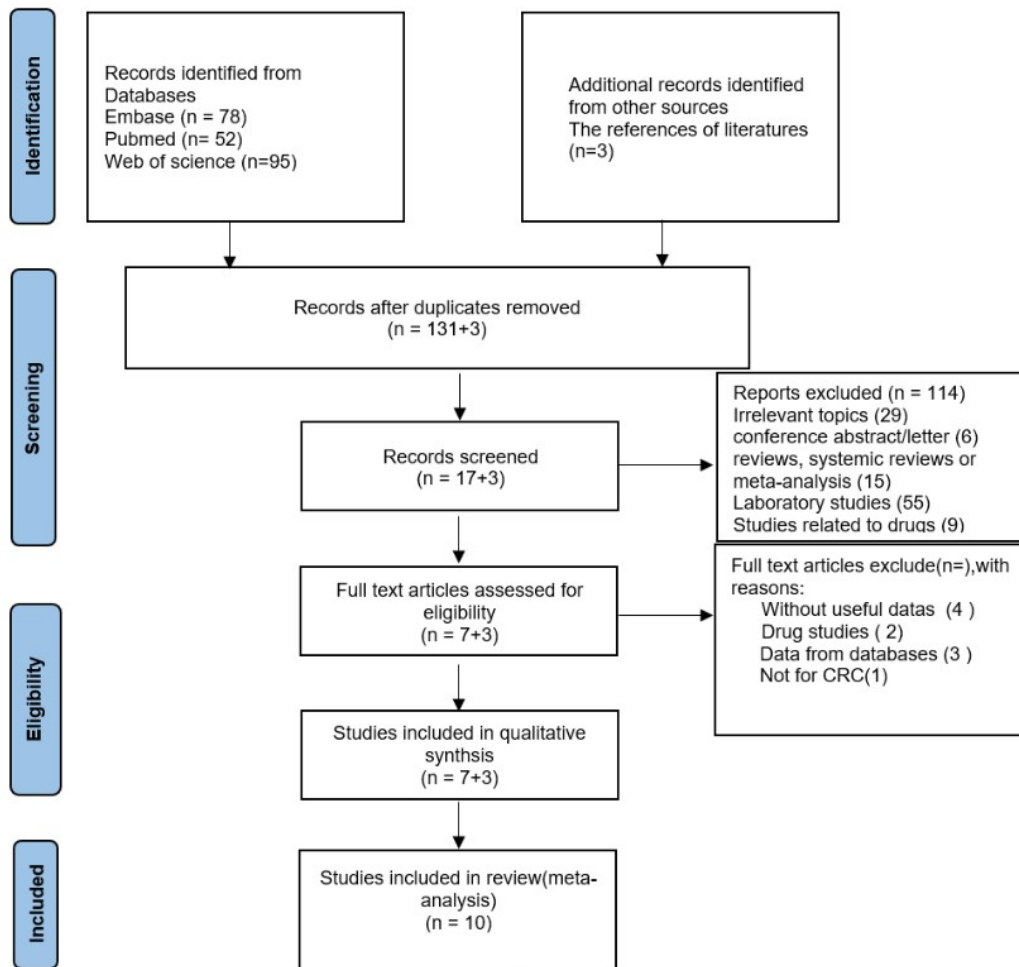


Fig. 1. Flow diagram of the study selection process.

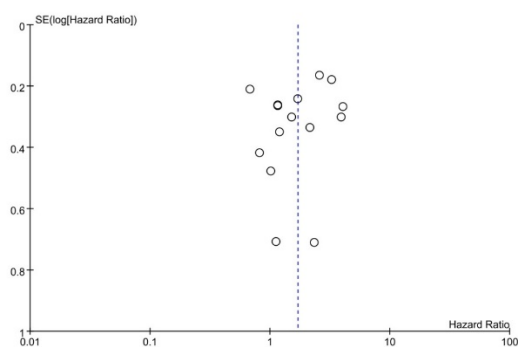


Fig. 2. Funnel plot of publication bias in the included studies.

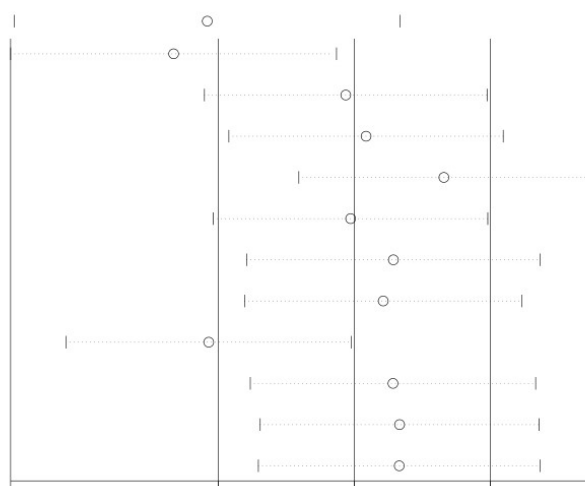


Fig. 3. Sensitivity analysis of the included studies.

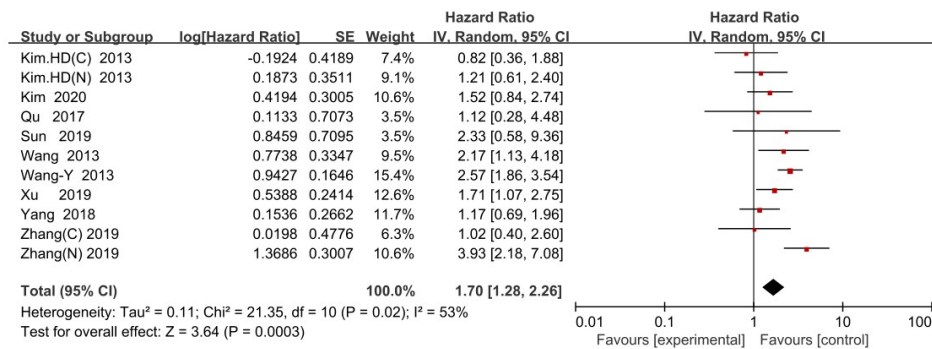
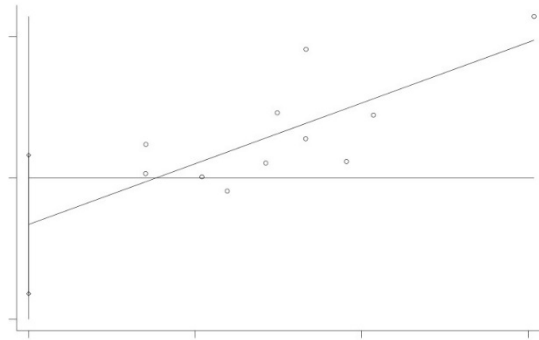


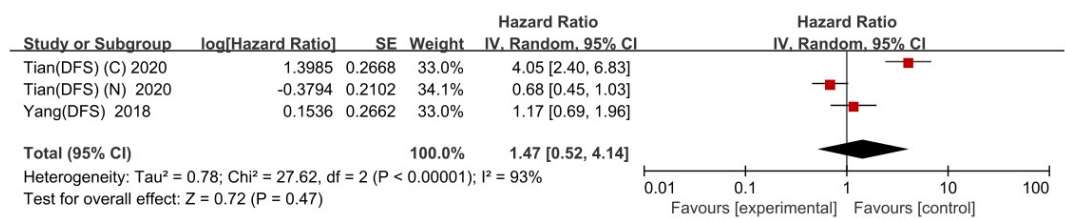
Fig. 4. Forest plot of HR for the association between YAP1 overexpression and OS.

Table 1. Subgroup analysis of the meta-analysis

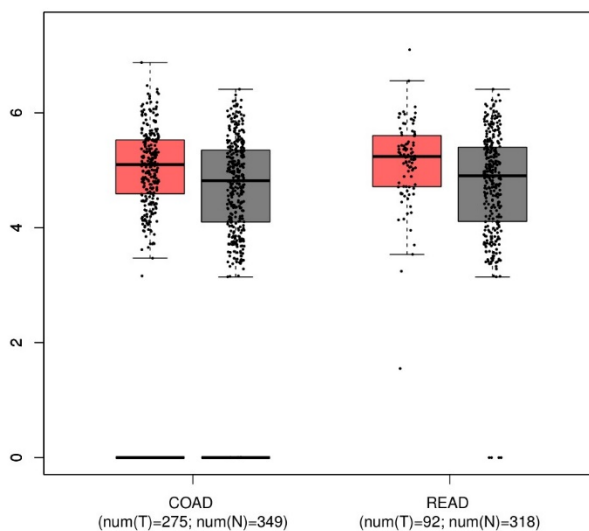
Analysis	HR (95 %)	p-value	Heterogeneity	
			I-square	p-value
OS				
<i>Ethnicity</i>				
Chinese	1.94 (1.40-2.69)	0.0001	52 %	0.04
Korean	1.23 (0.83-1.82)	0.31	0 %	0.49
<i>Test method</i>				
IHC	1.65 (1.17-2.33)	0.005	62 %	0.007
IHC + other methods	1.77 (1.13-2.77)	0.01	0 %	0.68
<i>Subcellular location</i>				
Cytoplasm	0.90 (0.49-1.68)	0.75	0 %	0.74
Nucleus	2.07 (1.29-3.31)	0.003	60 %	0.06
<i>Follow-up time</i>				
About 60 months	1.89 (1.30-2.73)	0.0008	0 %	0.81
Over 100 months	1.92 (0.88-4.20)	0.1	74 %	0.02
<i>Analysis type</i>				
Univariate análisis	1.61 (0.60-4.31)	0.34	0 %	0.46
Multivariate analysis	1.70 (1.24-2.31)	0.0009	62 %	0.008



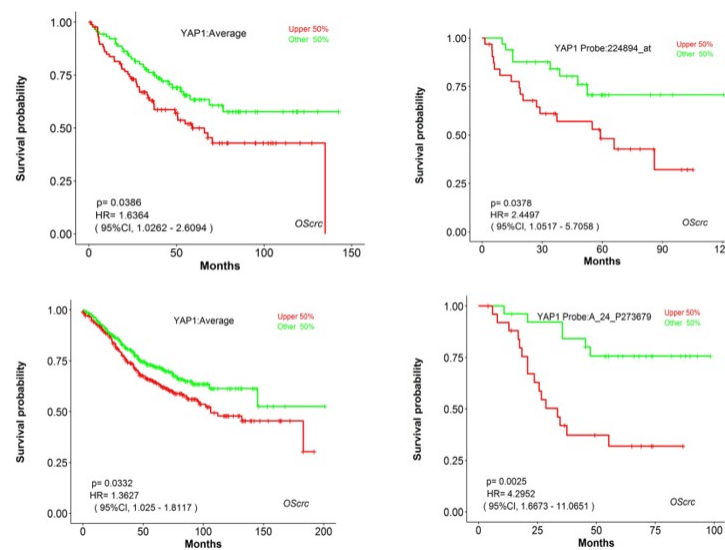
Supplementary Fig. 1. Egger's test of publication bias for include studies.



Supplementary Fig. 2. Forest plo of HR for association between YAP1 over-expression and DFS.



Supplementary Fig. 3. Over-expression of YAP1 in CRC in GEPIA.



Supplementary Fig. 4. Survival curves of YAP1 in LOGpc.

Supplementary Table 1. The characteristics of including articles

First author	Publish year	Country	Sample number	Age (year)		Location	No of YAP1 Over-expression	Detected-methods	Follow-up time (month)	TNM Stage	Outcome	HR (95 % CI)	NOS score
				< 60	≥ 60								
Y. Wang	2013	China	139	57	82	N	73 (52.5 %)	IHC	NA	I-IV	OS	U: 1.907 (1.104-3.293)	7
												M: 2.567 (1.859-3.546)	
L. J. Wang	2013	China	168	85	83	C/N	122 (72.6 %)	IHC	60	I-IV	OS	U: 1.617 (1.151-2.273)	8
												M: 2.168 (1.125-4.179)	
D. H. Kim	2013	Korea	144	61	83	N	N:49 (34.3 %)	IHC	NA	I-IV	OS	M: C(+):0.825 (0.363-1.877)	7
							C:64 (44.4 %)					M: N(+):1.206 (0.606-2.400)	
C. Qu	2017	China	90	51	39	NA	62 (68.9 %)	IHC	NA	I-IV	OS	U: 1.12 (0.28-4.56)	7
R. Yang	2018	China	145	23	31	NA	54 (37.2 %)	IHC	NA	NA	OS	U: 3.067 (1.961-4.798)	7
												M: 1.166 (0.698-1.949)	
											DFS	U: 3.034 (1.956-4.705)	
												M: 1.166 (0.692-1.963)	
Z. Q. Sun	2019	China	83	36	47	NA	59 (71.1 %)	IHC+qPCR	60	I-IV	OS	U: 2.33 (0.58-9.38)	8
Z. H. Xu	2019	China	116	NA		N	76 (65.5 %)	IHC+qPCR+WB	60	NA	OS	U: 2.128 (1.349-3.356)	9
												M: 1.714 (1.068-2.752)	
S. Zhang	2019	China	206	61	145	N/C	175 (85.0 %)	IHC	129	I-IV	OS	C(+)/N(+) 1	9
												U: C(-)/N(-) 5.85 (3.48 to 9.85)	
												M: C(-)/N(-) 3.93 (2.18 to 7.07)	
												U: C(+)/N(-) 1.13 (0.44 to 2.89)	
												M: C(+)/N(-) 1.02 (0.40 to 2.63)	
												U: C(-)/N(+) 5.85 (3.48 to 9.85)	

												M: C(-)N(+) 3.93 (2.18 to 7.07)	
J. Y. Kim	2020	Korea	295	89	206	NA	138 (46.8 %)	IHC	50 (1-147)	Tis-IV	OS	U: 2.108 (1.239-3.584)	7
												M: 1.521 (0.844-2.741)	
T. Q. Dong	2020	China	919	NA	C		462 (50.3 %)	IHC	NA	I-III	DFS	U: C(+)N(-) 3.891 (2.758-5.490)	7
												M: C(+)N(-) 3.255 (2.290-4.627)	
												U: C(-)N(+) 0.684 (0.453-1.031)	
											DSS	U: C(+)N(-) 4.291 (2.545-7.236)	
												M: C(+)N(-)4.049 (2.400-6.830)	

CI: confidence interval; DFS: disease-free survival; DSS: disease-specific survival; HR: hazard ratio; IHC: immunohistochemistry; OS: overall survival; N: nucleus; C: cytoplasm; NA: not told; (+)YAP1: over-expression; (-)YAP1: lower-expression; qPCR: quantitative real-time polymerasechain reaction; WB: Western Blot; U: univariate analysis; M multivariate analysis.

Supplementary Table 2. Association between YAP1 and DFS in CRC patients in GEPIA and LOGpc

Dataset	No. of YAP1 expression		DFS	
	High	Low	HR	<i>p</i> -value
GSE17536	87	87	1.4607	0.1306
GEPIA	181	180	1.1	0.79
GSE24551_GPL11028	80	80	0.9551	0.8568
GSE17537	28	27	1.0531	0.9109
GSE29623	26	27	0.46	0.2753
GSE38832	46	46	2.243	0.2552
GSE41258	86	85	1.1005	0.6296
GSE31595	18	19	1.3062	0.6338
GSE24551_GPL5175	80	80	0.6916	0.1618
GSE87211	46	46	0.4342	0.1223

No.: numbers of patients with YAP1 expression; High: higher expression of YAP1; Low: lower expression of YAP1; HR: hazard ratio.

