YAP1 protein expression has variant prognostic significance in SCLC stratified by histological subtypes

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Research

Keywords: Yes-associated protein 1 (YAP1), small cell lung cancer (SCLC), combined SCLC (C-SCLC), prognosis, biomarker

Posted Date: January 19th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-147852/v1

Abstract

Background

Recently, expression of Yes-associated protein 1 (YAP1), a nuclear effector of an inactivated HIPPO pathway, has been identified as one of four molecular subtypes of SCLC defined by the predominant transcriptional regulatory mechanism. However, the clinicopathological relevance and prognostic significance of YAP1 expression in SCLC stratified by histological subtypes has not been systematically reported to date.

Methods

Tumor sections and corresponding formalin-fixed paraffin-embedded (FFPE) samples of 297 SCLC patients were retrieved from the pathological specimen repository and were subsequently reviewed by pathologists. Forty-six C-SCLCs (15.5%) and 251 P-SCLCs (84.5%) were identified respectively. YAP1 expression was examined by immunohistochemistry (IHC) and assessed semi-quantitatively on tumor tissue array (TMA). Propensity score was used to match C-SCLCs and P-SCLCs in a ratio of 1 to 2 to balance age, gender, tumor stage and treatment methods. Finally, 46 C-SCLCs and 92 P-SCLCs were included for prognostic analysis.

Results

The positive rate of YAP1 expression was significantly higher in C-SCLCs than P-SCLCs before matching (52.2% vs 29.1%, $P = 0.004$). After matching by propensity score, the prescribed clinical parameters were well balanced between P-SCLCs and C-SCLCs. Expression of YAP1 was associated worse overall survival (OS) (5- year OS%, 39.0% vs. 74.9%, $P = 0.013$) and was an independent risk factor for OS (HR = 2.93, 95% CI: 1.01–8.51; $P = 0.048$) exclusively in C-SCLC. Univariate survival analysis in subgroups of different clinical variables also confirmed the prognostic impact of YAP1 was most significant in C-SCLC. But for P-SCLCs, expression of YAP1 showed no prognostic impact.

Conclusions

Expression of YAP1 in small cell components of C-SCLC was significantly higher than that in P-SCLC. Besides, it served as an unfavorable predictor for OS in C-SCLC but not in P-SCLC, which suggested different entities of small cell components with variant YAP1 expression and potential different targetable oncogenic pathway between C-SCLC and P-SCLC.

Background

Lung cancer is one of the most lethal malignancies in China,[1] of which small cell lung cancer (SCLC), the most aggressive histological type of lung cancer, accounts for 15%–20%. [2,3] SCLC is a high-grade neuroendocrine carcinoma of lung with limited therapeutic options and especially high mortality rate. Most of the SCLC are pure small cell lung cancer (pure SCLC, P-SCLC), while some can be combined with additional components of any histological types of non-small cell lung cancer (NSCLC), which is defined as combined small cell lung cancer (combined SCLC, C-SCLC).[4] The reported prevalence of C-SCLC is variable, ranging from 2–28% of all SCLC cases in different studies, which might be influenced by different methods for sampling.[5–7] Previous studies have shown that the clinical characteristics of C-SCLC do not differ significantly from those in patients with P-SCLC.[5,7,8] In addition, C-SCLCs have been treated based on SCLC guidelines,[9] similarly with a platinum agent and etoposide with or without thoracic radiation, after adjustments for tumor extent or stage for over three decades.[10] Therefore, to date, SCLC has been regarded as a 'homogenous' disease with little documented inter-tumor heterogeneity with respect to histology or molecular biology in clinical practice.[10] However, it has been reported that C-SCLC patients have lower response rates to chemotherapy compared to P-SCLC patients in some studies.[11,12] These results indicated histological subtypes have an impact on clinical management of SCLC and we can no longer regard SCLC as a single disease entity.[13] Besides, the difference of molecular biological characteristics between different histological subtypes in SCLC is worth further exploring to guide more effective and individualized therapy.

Recently, expression of YAP1 together with achaete-scute family bHLH transcription factor 1 (ASCL1), neuronal differentiation 1 (NEUROD1) and POU class 2 homeobox 3 (POU2F3) was identified as one of four molecular subtypes of SCLC defined by the predominant transcriptional regulatory mechanism operating in the tumor cells. However, the clinicopathological relevance and prognostic significance of YAP1 expression in SCLC has not been systematically reported to date. YAP1 is a downstream nuclear effector of the inactivated Hippo signaling pathway, which is essential for regulating cell proliferation, apoptosis, stem/progenitor cell expansion and organ growth and is considered as a conserved tumor suppressor pathway.[14–16] In case of an inactivated HIPPO pathway, Dephosphorylated YAP1 translocate into nucleus and promote the expression of genes associated with cell proliferation, reprogramming, stemness, epithelial-mesenchymal transition (EMT) and anti-apoptosis by acting as a coactivator together with other transcriptional regulators.[16–19] Recent studies have shown that YAP1 overexpresses in nucleus and acts as an oncoprotein in a broad range of human carcinomas, including NSCLC, gastric cancer, colorectal cancer, hepatocellular carcinoma, ovarian cancer and pancreatic cancer..[20–26] Furthermore, overexpression of YAP1 in nucleus is recognized as a poor prognostic marker in hepatocellular carcinoma, gastric cancer, colorectal cancer and NSCLC.[25,27–30] However, its tumor-suppressive roles were also demonstrated in some cancers such as breast cancer, hematological malignancies (leukemia and multiple myeloma) and SCLC.[31–34] Therefore, it seems that YAP1 exerts both oncogenic and tumor-suppressive activities in a context-dependent manner..[33] Importantly, YAP1 is identified as a tumor marker associated with sensitivity to drugs in various cancers including NSCLC and SCLC. In NSCLC cell lines, overexpression of YAP1 promoted resistance to both chemotherapeutic drugs

paclitaxel and cisplatin and targeted drugs erlotinib / gefitinib.[35–38] However, in SCLC, the impact of YAP1 expression on tumor chemosensitivity seems elusive and paradoxical. One study showed that SCLC cell lines with high YAP1 expression were more resistant to cisplatin and among patients receiving adjuvant chemotherapy, YAP1 positive cases had a significant shorter survival than YAP1 negative cases, but for patients without adjuvant chemotherapy, YAP1 positive patients had a better prognosis,[39] suggesting a potential co-existing roles as a tumor suppressor and a drug-resistant molecule. Nevertheless, another study which analyzed the data on the response to 526 anticancer agents in 61 SCLC cell lines showed SCLC cell lines with high YAP1 expression had higher sensitivity to etoposide and topotecan which were widely used for SCLC and were more sensitive to mechanistic target of rapamycin kinase (mTOR) and polo like kinase (PLK) inhibitors. Therefore, the author thought SCLC of the YAP subgroup may be more responsive to chemotherapy or targeted therapies.[40] Due to the complex signal network YAP1 involved in and dual role either as a tumor promoter or a tumor suppressor, the clinicopathological relevance of YAP1 in SCLC remain to be investigated. Besides, C-SCLC is a more biologically complex tumor consisting of both small cell and non-small cell components, in which YAP1 may have different impacts. Our study compared the expression of YAP1 between P-SCLC and C-SCLC mainly in the small cell components and explore its clinicopathological relevance respectively, aiming at demonstrating the difference of molecular biological characteristics and its impact on treatment and prognosis between P-SCLC and C-SCLC.

Materials And Methods

Patient selection and histological identification

Three hundred and forty-three patients who received a surgery and diagnosed as SCLC in Cancer Hospital, Chinese Academy of Medical Science (CHCAMS) between 2005 and 2016 were initially included. Clinical data including age, gender, smoking history, tumor laterality, TNM stage, stage by Veterans Administration Lung Study Group (VALSG), operation means and post-surgery treatment methods were extracted from medical record system. Forty-six patients without complete follow-up information were excluded. Tumor sections and corresponding formalin-fixed paraffin-embedded (FFPE) samples of the remaining 297 SCLC patients with complete medical records and follow-up information were retrieved from the pathological specimen repository of CHCAMS and were subsequently reviewed by one senior pathologist (Lin Yang) and two junior pathologists (Li Liu and Xin Wang). And the histological subtype was identified and confirmed independently according to the 2015 World Health Organization classification of lung tumors.[41] As for the combined pathology, we considered presence of at least 10% large cell for combined small cell and large cell carcinoma diagnosis. For small cell/squamous carcinoma or small cell/adenocarcinoma, presence of any amount of non–small-cell component was considered diagnostic.[7] Based on this criterion, 46 and 251 samples were identified as C-SCLC and P-SCLC respectively. We used propensity score to match C-SCLCs and P-SCLCs in a ratio of 1 to 2 with a caliper 0.2 to balance age, gender, tumor stage and treatment methods. Finally, 46 C-SCLCs and 92 P-SCLCs were included for analysis of the prognostic significance of YAP1 expression in different histological subtypes (P-SCLC vs. C-SCLC). Propensity score matching (PSM) was performed by R

software (version 3.6.3). This study was approved by the Ethics Committee and Institutional Review Boards of CHCAMS (NO.20/234-2430) and all patients were exempt from an informed consent due to the retrospective nature of the study.

Tumor section review and evaluation of pathological characteristics

Besides the histology identification, we further observed and recorded other pathological characteristics including status of lymph nodes (positive or negative), presence or absence of pleural invasion, bronchus invasion, vascular invasion, nerve invasion, tumor thrombosis and phenomenon of spread through air spaces (STAS), and proportion of necrosis, fibrosis and tumor infiltrating lymphocytes (TILs). The proportions of fibrosis/necrosis/TILs were defined as the ratio of the area of fibrosis or necrosis to the total area of the tumor section. The proportion of fibrosis or necrosis was defined as the ratio of the area of fibrosis or necrosis to the total area of the tumor section. And the denominator used to determine the percentages of TILs is the area of tumor parenchyma. The proportions of TILs were based on a full assessment of average area of TILs within the tumor area from all selected sections as a previous study described.[42]

Tissue array construction and immunohistochemical (IHC) staining

The archived paraffin blocks corresponding to 297 SCLC cases included were retrieved and constructed into tissue microarrays (TMAs) (1.5mm x 2 punctures per donor block). Serial 4um tissue sections were cut and stained with hematoxylin and eosin (H&E). Staining intensity of diagnostic markers (CgA, CD56, Syn, Ki-67) for SCLC and (P63, P40, TTF-1, NapsinA) for NSCLC reported on the original pathology report sheet were referred for histological identification when morphology was equivocal for diagnosis. The rabbit monoclonal antibody against human YAP1 (1:80; ab52711, Abcam) was used for immunostaining of YAP1. IHC staining was completed on the fully automatic Roche immunohistochemical instruments (Roche Diagnostics, Shanghai, China) according to the recommended standard protocols. YAP1 expression was scored based on the percentage of cell nuclei that stained positively: less than 10% or no staining, (–); 10% to 25%, (+); 25%-50%, (++); more than 50%, (+++). The expression of YAP1 was defined as positive when 10% of the tumor cell nuclei or greater were stained (scores 1+, 2+ or 3+) and negative when less than 10% were stained (scores –).

Follow-up strategy

The follow-up was conducted by regular patient visits or telephone calls and completed until February 28, 2019. In general, patients with limited stages were recommended for outpatient review every 3 months in year 1 to 2, every 6 months in year 3, and annually thereafter and patient with extensive stages were recommended for outpatient review every 2 months in year 1, every 3 to 4 months in year 2 to 3, every 6 months in year 4 to 5 and annually thereafter as NCCN guideline instructed.[43] The follow-up periods and intervals were then determined according to tumor status and treatment recommended by physicians. The median follow-up time was 47.2 months for overall cohort, 46.5 months for C-SCLCs and 47.8 months for P-SCLCs respectively. Relapse-free survival (RFS) is defined as the time durance between the

start of surgery and the observation of local recurrence or distant metastasis of the tumor confirmed by imaging or biopsy of metastatic sites. In case of no recurrence during follow-up, the endpoint of RFS is the last follow-up or death. Overall survival (OS) is defined as the time from the date of surgery to death or the last follow-up (in case of no death). The primary endpoint of this study was OS, and second endpoint was RFS.

Statistical analysis

All statistical analysis was performed on SPSS software version 25.0. For continuous normal distribution variables, the mean ± standard deviation was calculated, and the Student's t test was applied to show the significance of difference between groups. For continuous abnormal distribution variables, the median and quartile were calculated, and the Wilcoxon rank sum test was applied to show the significance of difference between groups. For categorical variables, the percentage was calculated, and the Fisher's exact test or the Chi-square test was applied to determine the significance of difference. The Kaplan-Meier method was used to estimate and compare survival, with the log-rank test applied for significance testing. The Cox proportional hazards model was used for multivariate survival analysis. Both the univariate and multivariate analysis were performed in P-SCLCs and C-SCLCs separately. All statistical tests were bilateral and $P < 0.05$ was considered statistically significant. $P < 0.1$ was considered as a trend.

Results

Clinicopathological relevance of YAP1 expression

Firstly, to identify the clinicopathological relevance of YAP1 expression in SCLC, we compared the clinicopathological features between YAP1 positive and YAP1 negative in total 297 SCLC patients (Table 1). Overall, there were 97 (32.7%) YAP1 positive patients and 200 (67.3%) YAP1 negative patients. We found that YAP1 positive group contained more C-SCLC patients than YAP1 negative group (24.7% vs. 11.0%, $P = 0.004$, Table 1). Besides, the mean age was significantly higher in YAP1 positive patients than in YAP1 negative patients (55.90 ± 10.30 vs. 59.40 ± 8.83, $P = 0.004$, Table 1). And YAP1 positive group contained more males (81.4% vs. 65.5%, $P = 0.007$, Table 1). In addition, we found a correlation of YAP1 expression with smoking since a higher percentage of smokers was observed in YAP1 positive group (74.2% vs. 61.5%, $P = 0.042$, Table 1). There was no significant difference in other clinical parameters including tumor laterality, clinical stage, VALSG stage, treatment modes and patterns of relapse. As for pathological features, we found a significant higher percentage of necrosis in YAP1 positive group with median and quartile 30 [20, 40] vs. 20 [10, 30] in YAP1 positive group and negative group respectively (Table 1). Besides, there was a trend towards less incidence of nerve invasion in YAP1 positive patients (25.8% v. 37.0%, $P = 0.073$, Table 1). Consistent with these results, we observed that the positive rate of YAP1 was significant higher in C-SCLC (C-SCLC vs. P-SCLC, 52.2% vs. 29.1%, $P = 0.004$), male sex (male vs. female, 37.6% vs. 20.7%, $P = 0.007$), patients with age above 60 (age > 60 vs. age ≤ 60, 43.1% vs. 26.6%, $P = 0.005$) and smokers (yes vs. no: 36.9% vs. 24.5%, $P = 0.042$) (Fig. 1).

Table 1
Clinicopathological feature in 297 SCLC patients stratified by YAP1 expression.

Clinicopathological parameters	YAP1		
	Negative	Positive	*P**
	(n = 200)	(n = 97)	
Histological subtype (%)			0.004
P-SCLC	178 (89.0)	73 (75.3)	
C-SCLC	22 (11.0)	24 (24.7)	
Age (mean (SD))	55.90 (10.30)	59.40 (8.83)	0.004
Sex = Male (%)	131 (65.5)	79 (81.4)	0.007
Smoking = Yes (%)	123 (61.5)	72 (74.2)	0.042
Tumor laterality = Right (%)	107 (53.5)	44 (45.4)	0.233
Clinical stage (%)			0.487
I	54 (27.0)	33 (34.0)	
II	56 (28.0)	29 (29.9)	
III	83 (41.5)	32 (33.0)	
IV	7 (3.5)	3 (3.1)	
VALSG stage (%)			1.000
Limited stage	193 (96.5)	94 (96.9)	
Extensive stage	7 (3.5)	3 (3.1)	
Treatment (%)			0.312
S	24 (12.0)	11 (11.3)	
S + CTx	106 (53.0)	60 (61.9)	
S + CTx + RT	70 (35.0)	26 (26.8)	
Operation means (%)†			0.122
Lobectomy	163 (81.5)	73 (75.3)	
Pneumonectomy	12 (6.0)	8 (8.2)	
Wedge resection	9 (4.5)	10 (10.3)	
CTx before S = Yes (%)	20 (10.0)	7 (7.2)	0.522

Clinicopathological parameters	YAP1		
	Negative	Positive	*P**
	(n = 200)	(n = 97)	
CTx = Yes (%)	176 (88.0)	86 (88.7)	1.000
RT = Yes (%)	70 (35.0)	26 (26.8)	0.199
PCI = Yes (%)	45 (22.5)	21 (21.6)	0.987
Patterns of relapse (%) ‡			0.704
DM	46 (23.0)	26 (26.8)	
IR	26 (13.0)	12 (12.4)	
DM + IR	17 (8.5)	5 (5.2)	
No recurrence	103 (51.5)	49 (50.5)	
Lymph nodes = Positive (%)	129 (64.5)	54 (55.7)	0.180
Pleural invasion = Yes (%)	66 (33.0)	37 (38.1)	0.457
Bronchus invasion = Yes (%)	168 (84.0)	81 (83.5)	1.000
Vascular invasion = Yes (%)	165 (82.5)	84 (86.6)	0.464
Nerve invasion = Yes (%)	74 (37.0)	25 (25.8)	0.073
Tumor thrombosis = Yes (%)	107 (53.5)	48 (49.5)	0.599
STAS = Yes (%)	149 (74.5)	69 (71.1)	0.634
Necrosis (median [IQR])	20 [10, 30]	30 [20, 40]	< 0.001
Fibrosis (median [IQR])	20 [10, 20]	20 [10, 20]	0.934
TILs (median [IQR])	20 [10, 30]	20 [10, 30]	0.078

P- SCLC: pure small cell lung cancer; C-SCLC: combined small cell lung cancer; VALSG: Veterans Administration Lung Study Group; S, surgery; CTx, chemotherapy; RT, radiotherapy; PCI: prophylactic cranial irradiation; TILs: tumor infiltrating lymphocytes; STAS: spread through air spaces.

†22 patients were excluded because the surgical information was unavailable. ‡13 patients were excluded because the sites of recurrence was not recorded.

**P* < 0.05 is indicated by bold italics.

Combined components of C-SCLC and YAP1 expression in non-small cell components of C-SCLC

The non-small cell components in C-SCLC were mainly squamous cell carcinoma (SCC) and adenocarcinoma (ADC) with 19 cases (41.3%) and 18 (39.1%) cases respectively, followed by 4 (8.7%)

cases of large cell carcinoma, 2 (4.3%) cases of large cell neuroendocrine carcinoma, 1 (2.1%) case of carcinoid tumor, 1 case of carcinoid tumor combined with large cell neuroendocrine carcinoma, and 1 (2.1%) case of adenosquamous carcinoma. In addition to small cell components of C-SCLC, we further assessed the YAP1 expression in non-small cell components of C-SCLC. In 46 C-SCLC patients, YAP1 was found positive in 27 (58.7%) patients. Specifically, YAP1 positive rate was 83.3% (15/18), 58.0% (11/19) and 11.1% (1/9) in ADC, SCC and the rest of components respectively. Figure 3a showed a representative C-SCLC patient whose tumor consisted of small cell and ADC components and was found positive for YAP1 expression in both components (Fig. 3b).

Clinicopathological characteristic distribution before and after propensity score matching (PSM)

We used PSM to match C-SCLCs and P-SCLCs in a ratio of 1 to 2 to balance age, gender, tumor stage and treatment methods. Ninety-two cases of P-SCLC patients were successfully matched with 46 C-SCLC patients and the prescribed clinical parameters were well balanced between P-SCLC and C-SCLC cases. Before matching, the age of C-SCLC patients was higher than P-SCLC patients (59.65 ± 8.72 vs. 56.56 ± 10.12, $P = 0.053$), but the imbalance was negligible after matching with mean ± standard deviation of age 59.77 ± 8.11 in P-SCLC patients ($P = 0.937$). Other clinical parameters like gender, clinical stage, VALSG stage, treatment methods were also well balanced with $P > 0.05$ (Table 2). The detailed clinicopathologic features of 46 C-SCLC cases and 92 P-SCLC cases were shown in Table 2. However, YAP1 expression level was significantly higher in small cell components of C-SCLCs than in that of P-SCLCs before and after PSM (before PSM: median 1 vs. 0, $P = 0.00058$, Fig. 2a; after PSM: median 1 vs. 0, $P = 0.011$, Fig. 2b).

Table 2
The clinical parameters of combined SCLC and pure SCLC before and after matching.

Clinical parameters	Before matching			After matching		
	P-SCLC	C-SCLC	*P*	P-SCLC	C-SCLC	*P*
	(n = 251)	(n = 46)		(n = 92)	(n = 46)	
Age (mean (SD))	56.56 (10.12)	59.65 (8.72)	0.053	59.77 (8.11)	59.65 (8.72)	0.937
Age group (%)	164 (65.3)	24 (52.2)	0.124	50 (54.3)	24 (52.2)	0.952
≤ 60 years	164 (65.3)	24 (52.2)		50 (54.3)	24 (52.2)	
> 60 years	87 (34.7)	22 (47.8)		42 (45.7)	22 (47.8)	
Sex = Male (%)	175 (69.7)	35 (76.1)	0.486	67 (72.8)	35 (76.1)	0.837
Smoking = Yes (%)	159 (63.3)	36 (78.3)	0.074	62 (67.4)	36 (78.3)	0.259
Tumor laterality = Right (%)	125 (49.8)	26 (56.5)	0.498	46 (50.0)	26 (56.5)	0.588
Clinical stage (%)			0.469			0.984
I	78 (31.1)	9 (19.6)		19 (20.7)	9 (19.6)	
II	70 (27.9)	15 (32.6)		27 (29.3)	15 (32.6)	
III	95 (37.8)	20 (43.5)		42 (45.7)	20 (43.5)	
IV	8 (3.2)	2 (4.3)		4 (4.3)	2 (4.3)	
VALSG stage (%)			0.682			1.000
Limited stage	242 (96.4)	44 (95.7)		88 (95.7)	44 (95.7)	
Extensive stage	9 (3.6)	2 (4.3)		4 (4.3)	2 (4.3)	
Treatment (%)			0.485			0.958
S	32 (12.7)	3 (6.5)		6 (6.5)	3 (6.5)	
S + CTx	140 (55.8)	26 (56.5)		50 (54.3)	26 (56.5)	
S + CTx + RT	79 (31.5)	17 (37.0)		36 (39.1)	17 (37.0)	
Operation means (%)†			0.623			0.732
Lobectomy	200 (79.7)	36 (78.3)		75 (81.5)	36 (78.3)	
Pneumonectomy	18 (7.2)	2 (4.3)		7 (7.6)	2 (4.3)	
Wedge resection	15 (6.0)	4 (8.7)		6 (6.5)	4 (8.7)	

Clinical parameters	Before matching			After matching		
	P-SCLC	C-SCLC	*P*	P-SCLC	C-SCLC	*P*
	(n = 251)	(n = 46)		(n = 92)	(n = 46)	
CTx before S = Yes (%)	25 (10.0)	2 (4.3)	0.278	3 (3.3)	2 (4.3)	1.000
CTx = Yes (%)	219 (87.3)	43 (93.5)	0.339	86 (93.5)	43 (93.5)	1.000
RT = Yes (%)	79 (31.5)	17 (37.0)	0.576	36 (39.1)	17 (37.0)	0.951
PCI = Yes (%)	59 (23.5)	7 (15.2)	0.294	21 (22.8)	7 (15.2)	0.410

P-SCLC: pure small cell lung cancer; C-SCLC: combined small cell lung cancer; VALSG: Veterans Administration Lung Study Group; S, surgery; CTx, chemotherapy; RT, radiotherapy; PCI: prophylactic cranial irradiation.

†22 patients before matching and 8 patients after matching were excluded because the surgical information was unavailable.

Positive YAP1 expression was associated a worse prognosis in C-SCLC but not in P-SCLC

The follow-up period for the entire cohort after matching ranged from 4.0 to 166.6 months, and the median follow-up time was 47.2 months, 47.8 months, 46.5 months for entire cohort, P-SCLC cohort and C-SCLC cohort respectively. In total, 67 (48.6%) patients had relapsed and 52 (37.7%) patients deceased at the end of follow-up. During the follow-up, 27 (19.6%) patients were lost. The 5-year RFS rates were 51.9%, 54.0%, 47.6% and the 5-year OS rates were 63.3%, 66.4%, 56.7%, for entire cohort, P-SCLC cohort and C-SCLC respectively. The median RFS was 74.7 months, 85.7 months, 59.3 months and the median OS was 110.6 months, 110.6 months, NA for entire cohort, P-SCLC cohort and C-SCLC cohort respectively. No significant RFS ($P = 0.901$) and OS ($P = 0.700$) were observed between P-SCLC and C-SCLC (Figure S2). According to univariate survival analysis in the 92 P-SCLC patients, YAP1 expression not significantly correlated with RFS ($P = 0.863$, Figure S1a) or OS ($P = 0.728$, Fig. 4a). However, for 46 C-SCLC patients, YAP1 positive patients had a shorter RFS and OS compared with YAP1 negative patients (RFS, 25.2 vs. 97.7 months, $P = 0.366$, Figure S1b; OS, 38.8 vs. NA months, $P = 0.013$, Fig. 4b), with the 5-year RFS rate 42.3% vs. 54.4% ($P = 0.366$) and the 5-year OS rate 39.0% vs. 74.9%, $P = 0.013$) in YAP1 positive and YAP1 negative patients respectively, although the effect of YAP1 expression on RFS was insignificant ($P = 0.366$, Figure S1b).

Multivariate analysis identified positive YAP1 expression as an independent prognostic factor for C-SCLC

Variables including age group, gender, smoking history, TNM stage, VALSG staging, present or absent lymph node invasion, whether or not receiving prophylactic cranial irradiation (PCI) and YAP1 expression (positive or negative) were included in the Cox proportional hazard model for multivariate survival analysis in P-SCLC and C-SCLC separately. Positive YAP1 expression was identified as one of three independent risk factors for shorter OS in C-SCLC group (HR 2.93, 95% CI 1.01–8.51, $P = 0.048$, Fig. 5A).

The other two independent factors for OS of C-SCLC were VALSG stage (Extensive stage vs. Limited stage HR 19.91, 95% CI 2.75-144.29, *P* = 0.003, Fig. 5A) and presence of lymph node invasion (HR 4.09, 95% CI 0.93–17.98, *P* = 0.062, Fig. 5A). In P-SCLC group, only clinical stage was identified as an independent prognostic factor (*P* = 0.008, Fig. 5B) and YAP1 expression had no prognostic significance for OS.

Comparison of the effect of YAP1 expression on OS in different subgroup of entire cohort

We further compared OS between YAP1 positive (+) and YAP1 negative (-) patients in different subgroups of total 138 SCLC patients. Overall YAP1 (+) was an unfavorable indicator for OS, but the prognostic effect of YAP1 (+) was most significant in C-SCLC (HR 3.46, 95% CI 1.23–9.77, *P* = 0.019, Fig. 6). Besides, YAP1 (+) also associated with worse survival in patients with age ≤ 60 (HR 2.04, 95% CI 0.92–4.51, *P* = 0.077), current or former smokers (HR 1.62, 95% CI 0.88–2.97, *P* = 0.120), patients with lymph node invasion (HR 1.79, 95% CI 0.98–3.29, *P* = 0.059) and patients without receiving PCI (HR 1.66, 95% CI 0.93–2.97, *P* = 0.085, Fig. 6).

Discussion

In this study, to identify the clinicopathological relevance of YAP1 expression, we compared other clinicopathological parameters between YAP1 (+) patients and YAP1 (-) patients, and found that expression of YAP1 had correlation with histological subtype, age, gender and smoking history in SCLC with higher expression observed in patients with C-SCLC (*P* = 0.004), male sex (*P* = 0.007), age > 60 years (*P* = 0.005) and smoking history (*P* = 0.042). We further assessed the YAP1 expression in non-small cell components of C-SCLC and found that the YAP1 expression was commonly observed, especially in non-neuroendocrine carcinoma components like ADC (83.3%) and SCC (58.0%), with total positive rate 58.7%. These results were in accordance with the biological function of YAP1 which involved in inhibiting neuroendocrine differentiation.[39] However, the small cell components of C-SCLC were also commonly found positive YAP1 expression and expression of YAP1 was consistent with the non-small cell components and even higher than P-SCLC (52.2% vs. 29.1%, *P* = 0.004). This is reminiscent of a previous study which found that the histologic components of C-SCLC had high genetic concordance with approximately 75% of somatic mutations shared in small cell and non-small cell components, suggesting a common precursor of both components in C-SCLC. Our study supported this hypothesis since concordant YAP1 expression which was relatively rarely expressed in P-SCLC was observed between the two components with only 3/27 patients found positive YAP1 expressed exclusively in non-small cell components. Furthermore, positive expression of YAP1 was associated with worse prognosis in C-SCLC but not in P-SCLC, implicating that the small cell components in C-SCLC and P-SCLC might be of different entities with potential different origins, oncogenic and tumor-promoting mechanism which might be selectively targetable in C-SCLC. In addition, YAP1 expression could serve as a potential candidate biomarker for prognosis prediction exclusively in C-SCLC.

Previous studies have shown that compared with P-SCLC patients, C-SCLC patients have different response rates to chemotherapy indicating underlying different molecular mechanisms between

histological subtypes to regulate this response. YAP1 is a molecular involved in drug metabolism and its expression in SCLC cell lines is found influencing the sensitivity to chemotherapeutic drugs.[39,44] Besides, in SCLC patients receiving adjuvant chemotherapy, different OS was observed between YAP1 (+) and YAP1 (-) patients.[14] However, in the current study, we found the prognosis was not affected by YAP1 expression in P-SCLC patients. But in C-SCLC, YAP1 expression was indeed found correlated with prognosis since YAP1 (+) C-SCLC patients suffering a significant worse OS. Survival analysis in subgroups of different clinical variables also confirmed the prognostic impact of YAP1 was most significant in C-SCLC. This phenomenon might have translational significance in regarding the YAP1 as a candidate target for treatment of C-SCLC especially for patients with higher expression of this molecule. Since YAP1 is a vital downstream transcription factor in an inactivated Hippo pathway, overexpression of dephosphorylated YAP1 in nuclei suggests this pathway might be perturbed in C-SCLC which could be a potential mechanism explaining lower response rate to chemotherapy compared with P-SCLC. Further studies are needed to identify the specific mechanism for dysregulation of HIPPO signaling pathway in C-SCLC.

C-SCLC contains a various kind of non-small cell components with distinct molecular and pathologic characteristics, which might lead to different biological behaviors and treatment vulnerability from P-SCLC. Although C-SCLC is such a highly heterogeneous entity, the treatment choice of C-SCLC is universally consistent with the guidelines for SCLC, usually with a platinum agent and etoposide as common chemotherapeutic regimen.[9] Although some NSCLC chemotherapeutic regimens were also tried in C-SCLC, current retrospective data showed no significant difference in response rate, progression-free survival (PFS) and OS between patients receiving SCLC and NSCLC chemotherapeutic regimens, but the response rates to both regimen (30% in NSCLC regimen and 38.5% in SCLC regimen) were inferior to that observed in P-SCLC (50%-60%).[45] In this study, we speculated that overexpression of YAP1 in C-SCLC might be one of reasons contributing to the primary resistance to chemotherapy. In pre-clinical setting, with YAP1 inhibition, suppression of tumor progression and recovery of drug-sensitivities were observed in multiple cancer subtypes,[35,46,47] therefore YAP1 or perturbed HIPPO pathway might be an effective therapeutic target which deserved further testing in clinical trials. Besides, given the well-established role of YAP1 as a biomarker of resistance to a variety of drugs including chemotherapeutic agents and tyrosine kinase inhibitors (TKIs), combinational treatment strategies should be designed with YAP1 inhibition for treatment of this complex malignancy.

Our study represented first and largest research to investigate the clinicopathological relevance and prognostic significance of YAP1 expression in SCLC stratified by histological subtypes. For comparison of the prognostic significance of YAP1 expression, we applied propensity score matching (PSM) for matching P-SCLC and C-SCLC to balance gender, age, tumor stage and treatment methods since imbalance of these variables may lead to inter-patient bias which might further influencing prognostic analysis, therefore the reliability of the analytic results increased. Besides, the diagnosis of P-SCLC or C-SCLC was made and confirmed by postoperative pathological section which provided more comprehensive information than cytological or biopsy specimen, therefore increasing the detective rate

and accuracy of diagnosis of C-SCLC. Nevertheless, there were some limitation in our study. Firstly, due to the low incidence rate of C-SCLC patients, the number of studied populations was limited resulting a lack of statistical efficacy. Secondly, the assessment of YAP1 expression was based on TMAs which contained limited tissues thus the expression of YAP1 might be higher than current results. Thirdly, since only a small percentage of patients (6.5% in each group) did not receive postoperative adjuvant chemotherapy, we couldn't accurately assess the impact of YAP1 expression on prognosis in this subset of patients. Finally, due to the nature of a respective study, we could not identify the exact correlation of YAP1 expression with treatment response.

Conclusion

In conclusion, we found significant higher expression of YAP1 protein in small-cell components of C-SCLC than that of P-SCLC and positive expression of YAP1 was associated worse OS exclusively in C-SCLC. But for P-SCLC, no correlation of YAP1 expression with prognosis was observed. Our findings indicated the small cell components of P-SCLC and C-SCLC were of different entities with variant YAP1 expression and different prognostic vulnerability which further implicated potential different targetable oncogenic pathway between C-SCLC and P-SCLC. Further comprehensive and basic researches are needed to confirm our results and identify the underlying mechanism by which the YAP1 is overexpressed in C-SCLC and how it influences the treatment response and prognosis in C-SCLC.

Abbreviations

SCLC
small cell lung cancer
NSCLC
non-small cell lung cancer
P- SCLC
Q- pure small cell lung cancer
C- SCLC
D- combined small cell lung cancer
EMT
epithelial-mesenchymal transition
FFPE
formalin-fixed paraffin-embedded
IHC
immunohistochemistry
TMA
tumor tissue array
YAP1
yes-associated protein 1

mTOR
mechanistic target of rapamycin kinase
PLK
polo like kinase
ASCL1
achaete-scute family bHLH transcription factor 1
NEUROD1
neuronal differentiation 1
POU2F3
POU class 2 homeobox 3
CHCAMS
Cancer Hospital, Chinese Academy of Medical Science
VALSG
Veterans Administration Lung Study Group
STAS
spread through air spaces
TILs
tumor infiltrating lymphocytes
SCC
squamous cell carcinoma
ADC
adenocarcinoma
PSM
propensity score matching
OS
overall survival
RFS
relapse-free survival
PFS
progression-free survival
TKIs
tyrosine kinase inhibitors

Declarations

Ethics approval and consent to participate: This study was approved by the Ethics Committee and Institutional Review Boards of Cancer Hospital, Chinese Academy of Medical Science (CHCAMS) (NO.20/234-2430) and all patients were exempt from an informed consent due to the retrospective nature of the study.

Consent for publication: All authors are in agreement with the content of the manuscript for publication.

Availability of data and material: All patients included in this study came from Cancer Hospital, Chinese Academy of Medical Science (CHCAMS). Patients' specimens used for this study were retrieved from the pathological specimen repository of CHCAMS.

Competing interests: The authors declare that there are no conflicts of interest.

Funding: This work was funded by the Cancer Foundation of China, Beijing Hope Marathon Foundation [grant numbers LC2017A20] and the National Key Research and Development Program of China [grant number 2017YFC1308704, 2017YFC1311000 and 2017YFC1308700].

Authors' contributions: YL designed the study. LJL and XPY provided patients for study. WX, GYY, LL, ZJY, XTJ and DJY contributed to data collection and assemblage. GYY and LL performed data analysis. YL, GYY and WX were involved in the interpretation of data. GYY and WX drafted the manuscript. YL and WJC revised and corrected the manuscript. All authors contributed to the paper significantly.

Acknowledgements: We would like to acknowledge the Department Pathology for technical assistance with the experimental work.

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Figures

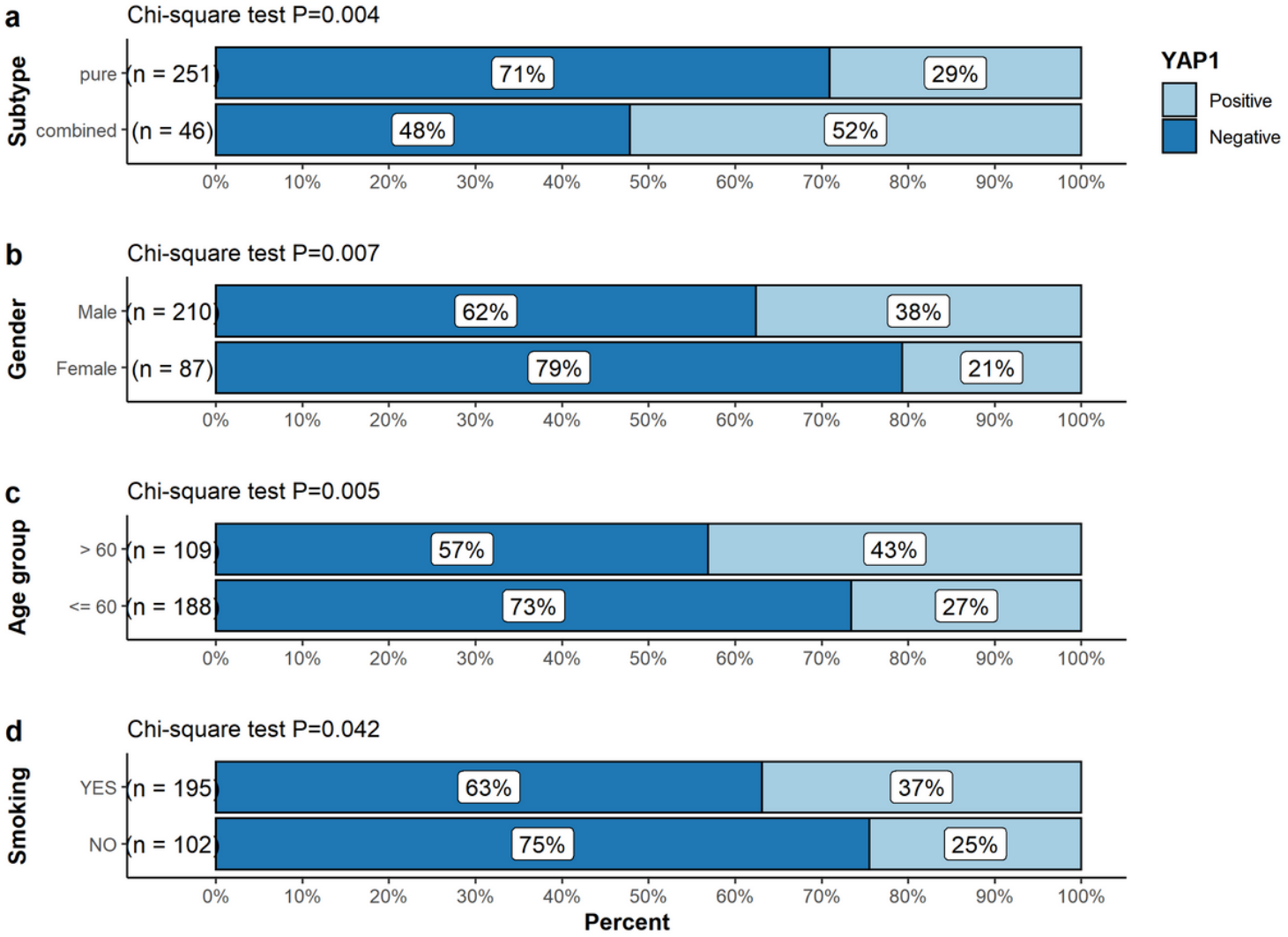

Figure 1

YAP1 positive rate was significantly imbalanced in different subgroups of subtypes (a), gender (b), age group (c) and smoking (d).

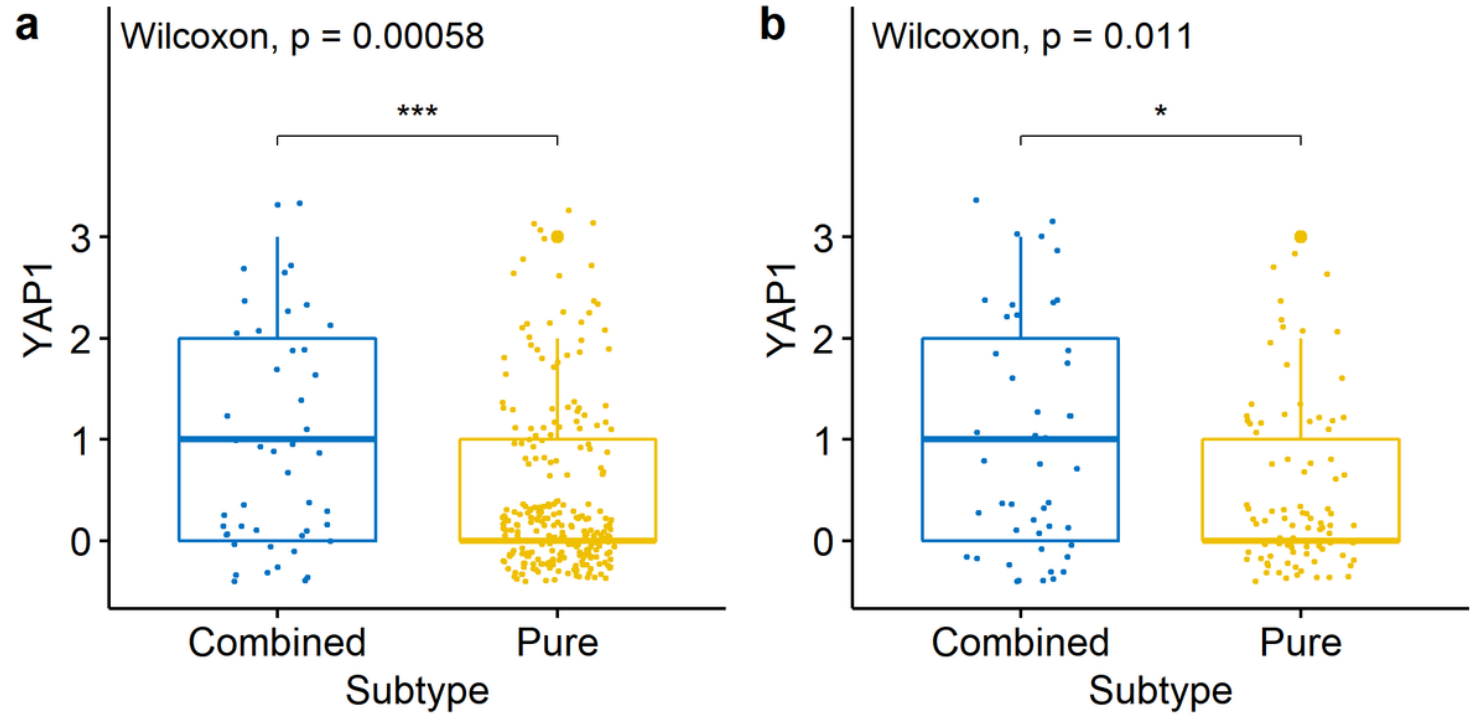

Figure 2

YAP1 expression was significantly higher in small cell component of combined SCLC than in pure SCLC before matching (a) and after matching (b). Wilcoxon rank sum test, P=0.00058 (a), P=0.011 (b).

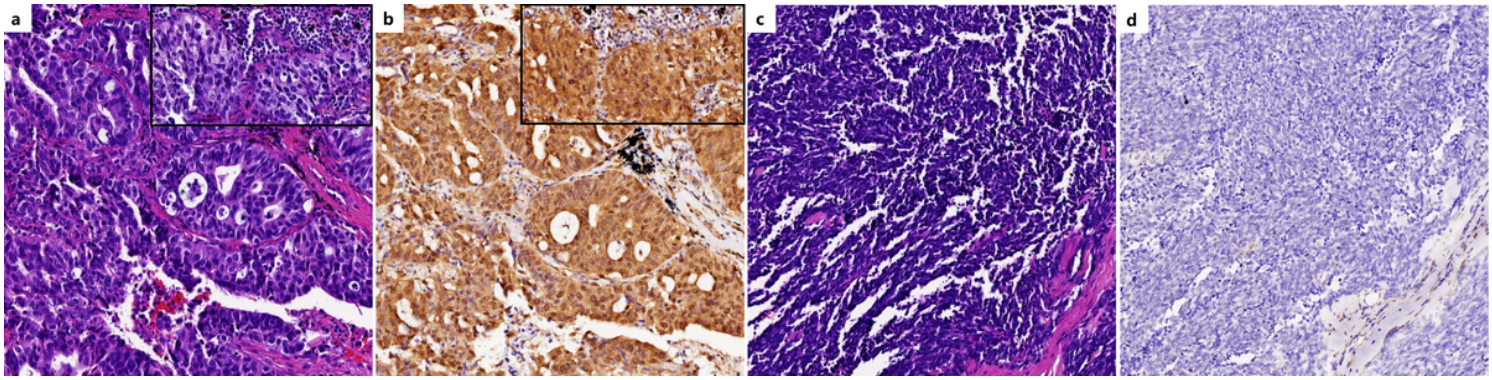

Figure 3

Representative slides of C-SCLC (a,b) and P-SCLC (c,d) stained with hematoxylin and eosin (H&E) (a,c) and immunostained with YAP1 antibody (b,d). The combined SCLC consisted of small cells components (top right corner) combined with adenocarcinoma (a) and was positive for YAP1 in both components (b). The pure SCLC consisted of pure small cell components (c) and was negative for YAP1 (d).

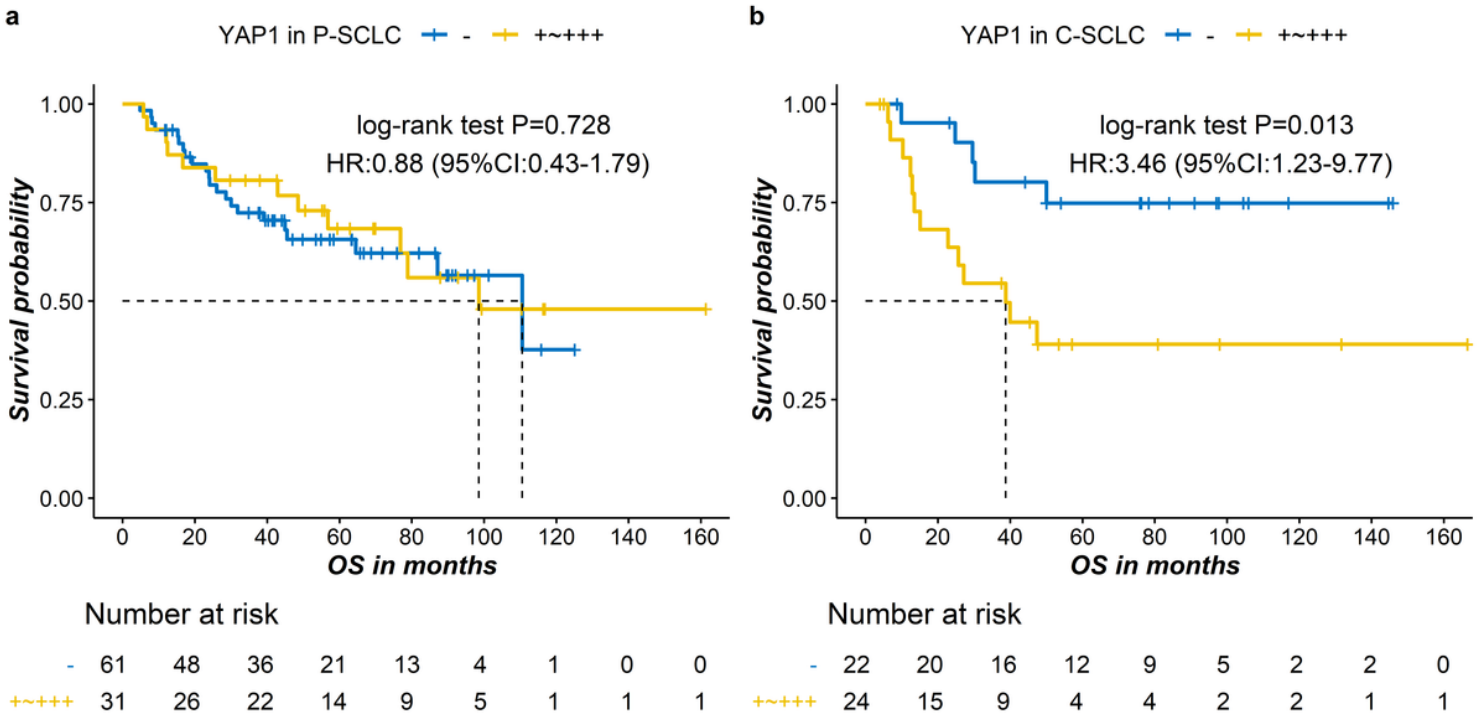

Figure 4

Comparison of overall survival (OS) between YAP1 positive and YAP1 negative patients with pure SCLC (a) and combined SCLC (b). For patients with combined SCLC, YAP1 positive patients had a significant worse OS than YAP1 negative patients (P=0.013) (b), but for pure SCLC patients, no significant different OS was observed in YAP1 positive and YAP1 negative patients (P=0.728) (a).

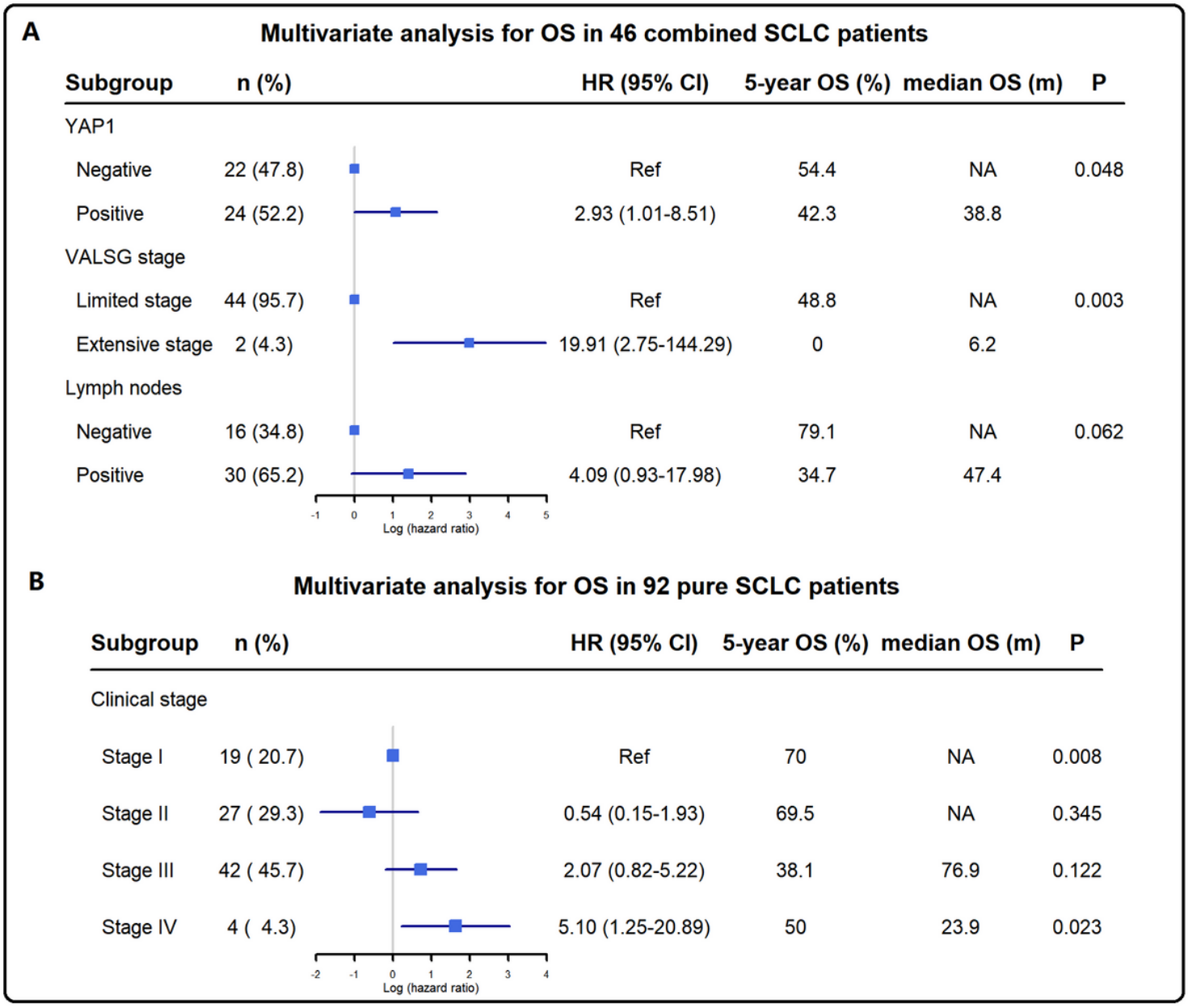

Figure 5

Multivariate analysis of overall survival (OS) in 46 combined SCLC (A) and 92 pure SCLC (B) by COX regression model. Multivariate analysis identified YAP1 (+) was an independent risk factor for OS in combined SCLC (HR 2.93, 95% CI 1.01-8.51; P=0.048) (A), but for pure SCLC, only clinical stage was identified as an independent prognostic factor (B).

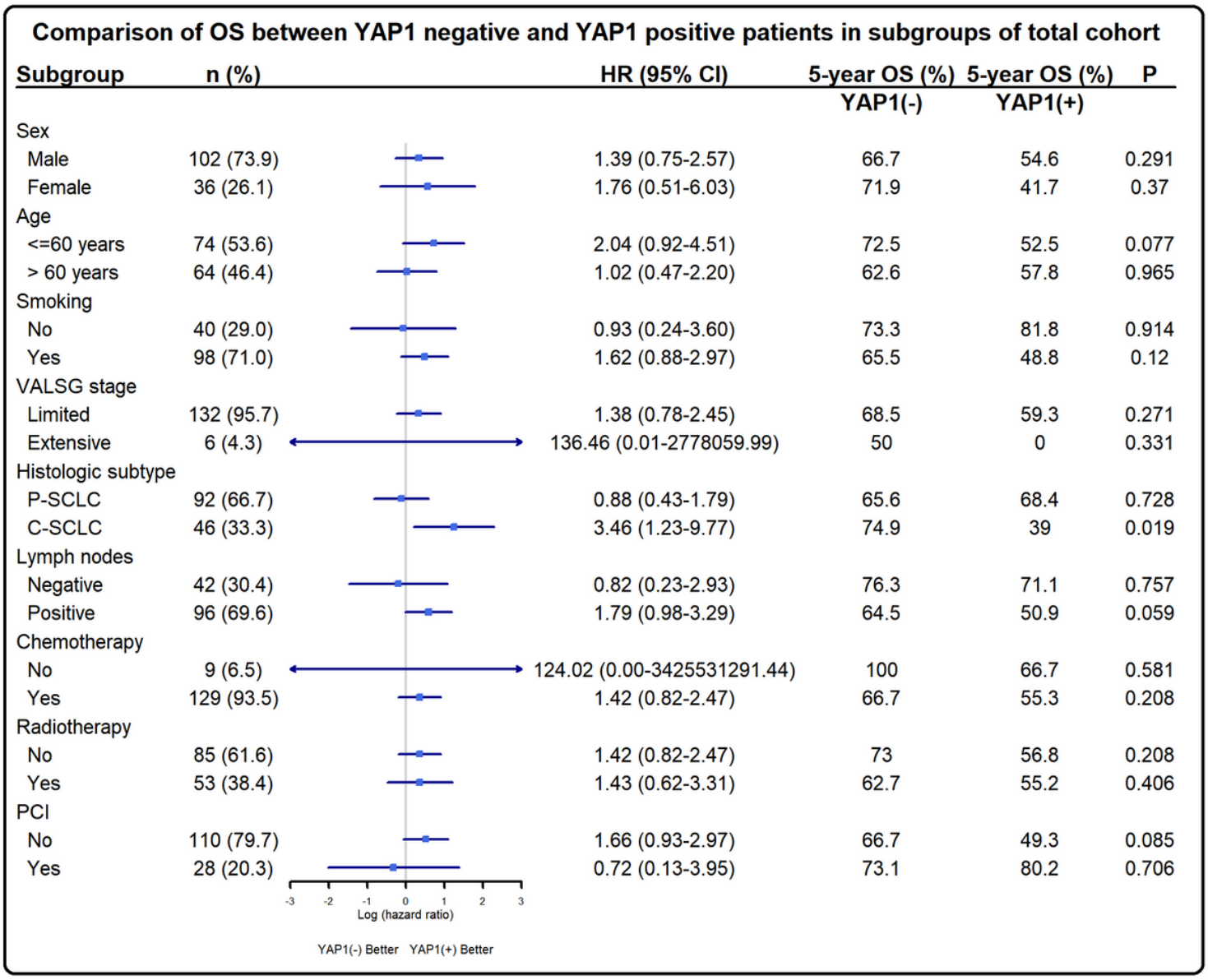

Figure 6

Comparison of overall survival (OS) between YAP1 (+) and YAP1 (-) patients in different subgroups of total 138 SCLC patients. Hazard ratio was calculated based on YAP (-) group as reference. The prognostic effect of YAP1 (+) was most significant in combined SCLC with 5-year OS 39.0% vs. 74.9% in YAP1 (+) and YAP1 (-) patients respectively (HR 3.46, 95% CI 1.23-9.77, P=0.019).

Supplementary Files

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