FOXO3 as a Novel Biomarker in Hepatocellular Carcinoma

Subjects: Gastroenterology & Hepatology | Oncology

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Hepatocellular carcinoma (HCC) represents one of the main causes of cancer-related death worldwide. The transcription factor forkhead box O3 (FOXO3) has been related to hepatic diseases and tumor progression, but the exact role played by FOXO3 on HCC still remains unclear. Recently, a novel systematic review with meta-analysis revealed the potential diagnostic and prognostic value of FOXO3 in this primary liver cancer type.

hepatocellular carcinoma | liver cancer | FOXO3 | diagnosis | prognosis | clinicopathological features | hcc | survival | invasion | forkhead box O3

1. Introduction

Liver cancer is the sixth most commonly diagnosed cancer and the third leading cause of tumor-associated death[1]. About 85% of liver cancer cases correspond to hepatocellular carcinoma (HCC)[1][2], an aggressive tumor with high incidence and mortality[3][4][5]. Unfortunately, only a slight percentage of patients are eligible for curative treatments[2][3][4] and the prognosis of HCC remains very poor[6]. Therefore, finding new functional biomarkers could improve HCC patient outcomes.

The forkhead box O subgroup (FOXO) of transcription factors is composed of FOXO1, FOXO3, FOXO4 and FOXO6[7][8][9]. FOXO3 has shown to exert physiological and pathological functions by controlling the transcription of key target genes involved in multiple cellular processes[9][10][11]. Nonetheless, contradictory reports about the role of FOXO3 expression in cancer are found in literature[7][10]. In regard to HCC, certain articles sustain that abnormal FOXO3 overexpression could constitute an unfavorable hallmark[12][13][14][15][16][17]. Otherwise, other studies defend the association of low FOXO3 expression with poorer HCC patient outcomes[18][19][20].

With the aim of clarifying the role played by FOXO3 on HCC as well as of investigating the potential value of FOXO3 as a new biomarker in HCC, a recent systematic review with meta-analysis (PROSPERO registration code: CRD42021237321) evaluated the correlation of FOXO3 expression with HCC pathogenesis, survival parameters and clinicopathological factors. It needs to be mentioned that the study followed the PRISMA guidelines[21], determining the quality of the selected investigations with the Newcastle-Ottawa scale (NOS) criteria[22], and performing the statitiscal analysis using previously reported methodology[23][24].

2. Main Findings

♦ 2.1. FOXO3 and HCC development

Pooled results from the comparison of FOXO3 expression between tumor tissues and healthy normal liver samples proved that elevated levels of FOXO3 significantly correlate with HCC pathogenesis (OR, 15.98; 95% CI, 1.96–130.02; $p = 0.01$) **(Figure 1a, Table 1)**.

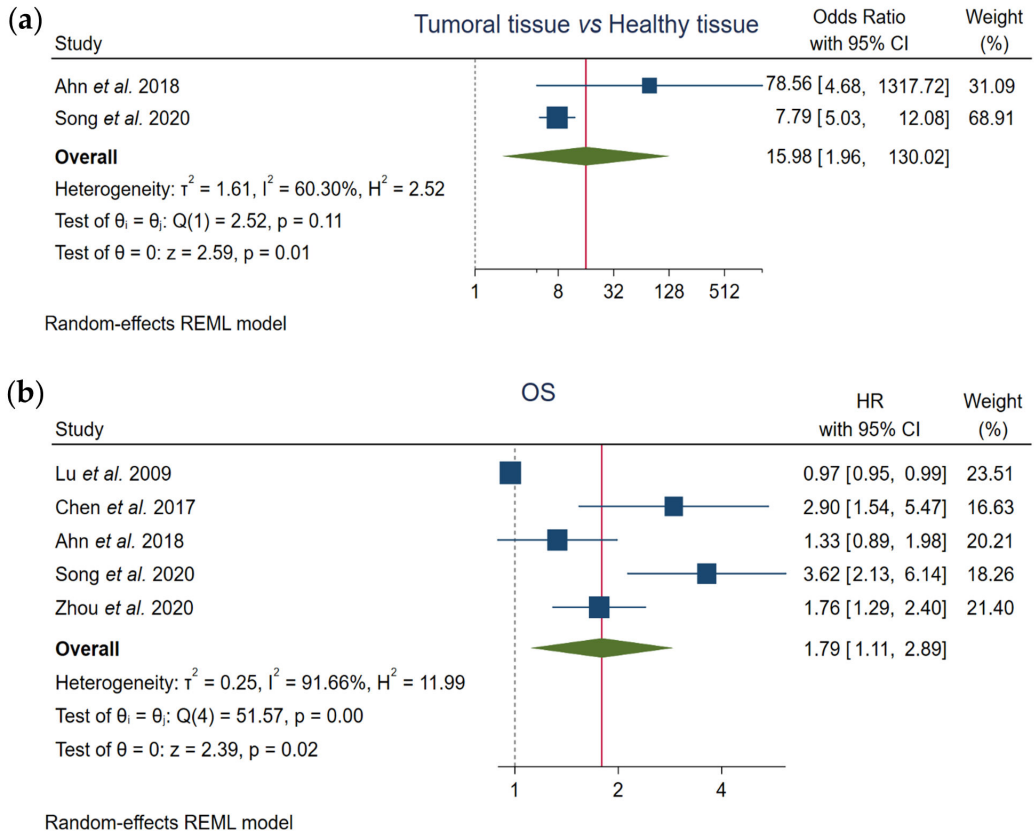


Figure 1. Assessment of the correlation of FOXO3 high expression with (**a**) tumor pathogenesis and (**b**) overall survival (OS) in HCC patients. CI, confidence interval; HR, hazard ratio; OS, overall survival; REML, Restricted Maximum Likelihood.

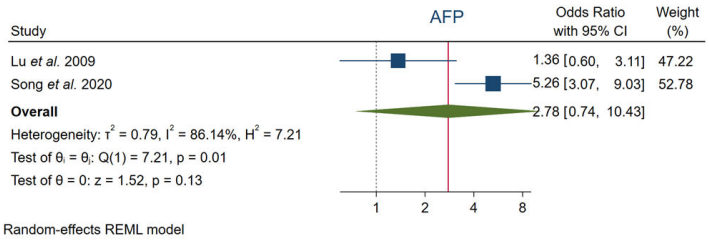
♦ 2.2. FOXO3 and OS

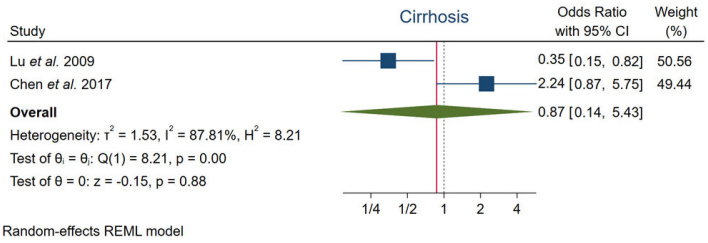
Based on the results of the total of the included articles, pooled data demonstrated that FOXO3 high levels significantly correlate with lower OS rates (HR, 1.79; 95% CI, 1.11–2.89; $p = 0.02$) **(Figure 1b, Table 1)**.

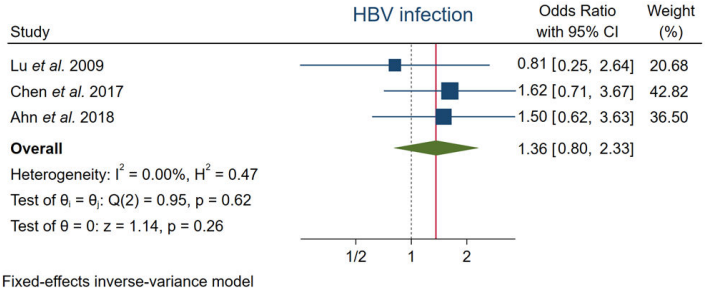
♦ 2.3. FOXO3 and Clinicopathological Features

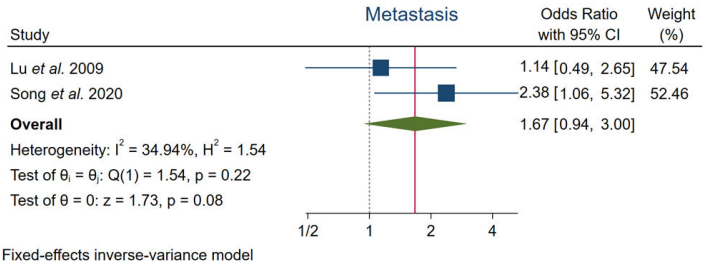
Initially, although all available data on clinicopathological factors were pooled and analyzed, the investigators did not observe any correlation of enhanced FOXO3 expression with different clinicopathological factors such as alpha-fetoprotein (AFP) levels, cirrhosis, gender, hepatitis B virus (HBV) infection, invasion, metastasis, tumor-

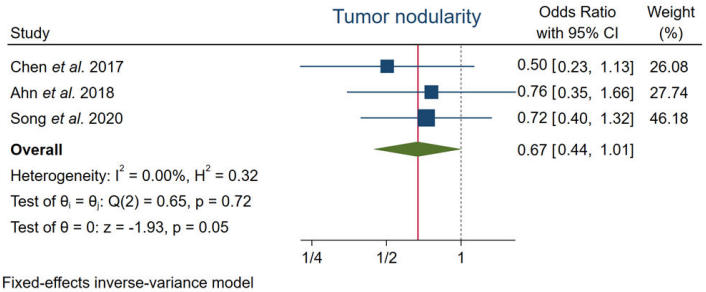
node-metastasis (TNM) staging, tumor nodularity and tumor size **(Figure 2, Table 1)**. However, certain parameters such as invasion showed an elevated heterogeneity among data. Therefore, subgroup analysis was subsequently conducted in order to investigate the heterogeneity causes and potentially find a significant correlation.

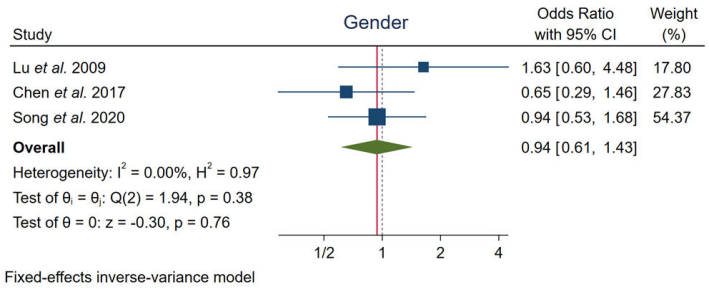


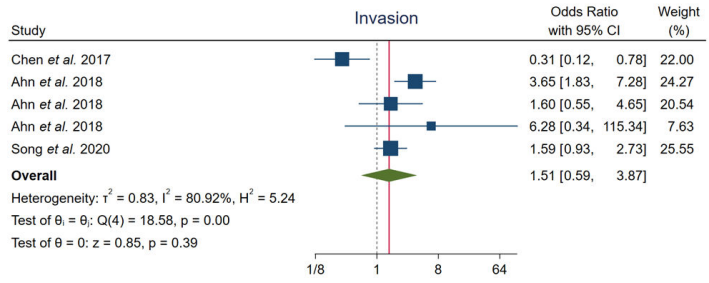


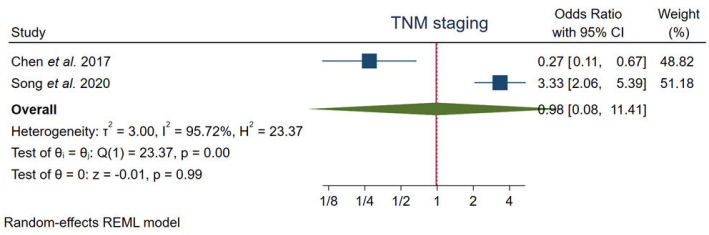












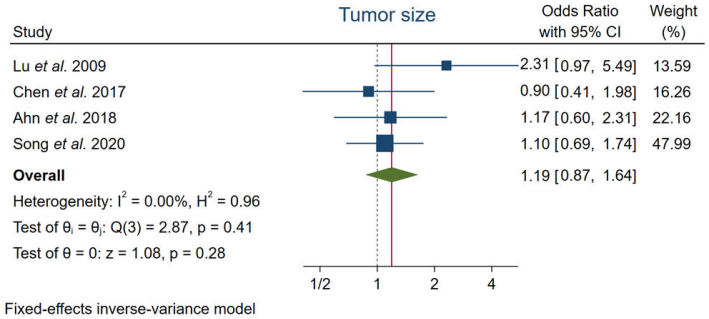


Figure 2. Analysis of the association between FOXO3 high expression and several clinicopathological features in HCC patients. AFP, alpha-fetoprotein; CI, confidence interval; HBV, hepatitis B virus; REML, Restricted Maximum Likelihood; TNM, tumor-node-metastasis.

Table 1. Evaluation of the association of enhanced FOXO3 levels with HCC pathogenesis, survival and clinicopathological features.

Parameter	Number of Studies (n)	Number of Cases (n)	Samples with High FOXO3 Expression (n)	High FOXO3 Expression (%)	Pooled OR or HR		Test for Heterogeneity		Model Used
					95% CI	*p*-Value	I^2	Q-Test *p*-Value	
HCC pathogenesis									
Tumoral tissue vs. Healthy tissue	2	672	402	59.82%	15.98 (1.96–130.02)	0.01	60.30%	0.11	REM
OS	5	1042	529	50.77%	1.79 (1.11–2.89)	0.02	91.66%	0.00	REM
Clinicopathological features									
AFP	2	346	153	44.22%	2.78 (0.74–10.43)	0.13	86.14%	0.01	REM
Cirrhosis	2	193	87	45.08%	0.87 (0.14–	0.88	87.81%	0.00	REM

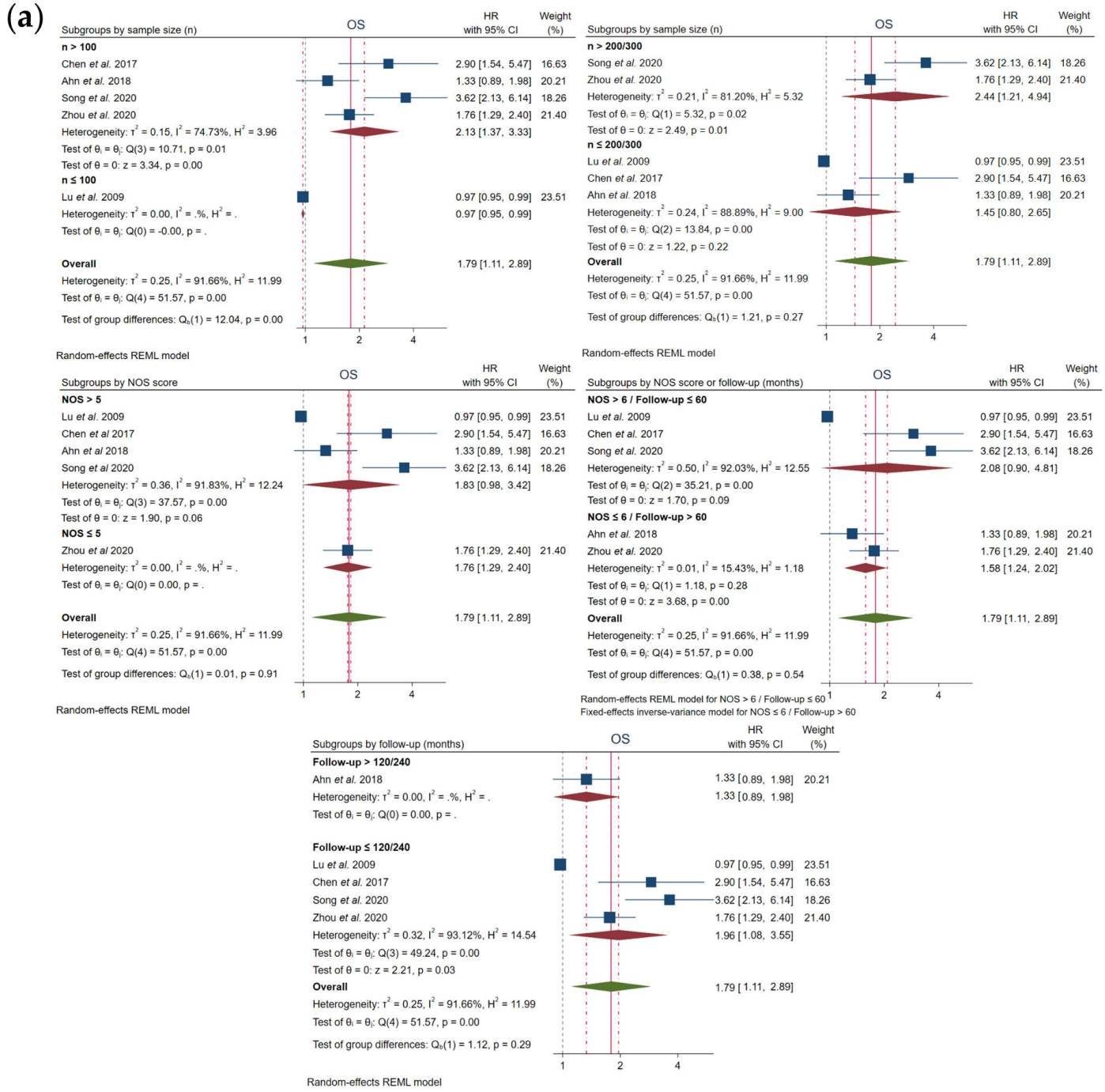
				5.43)				
Gender	3	507	211	41.62%	0.94 (0.61–1.43)	0.76	0.00%	0.38	FEM
HBV infection	3	378	207	54.76%	1.36 (0.80–2.33)	0.26	0.00%	0.62	FEM
Invasion	5	890	497	55.84%	1.51 (0.59–3.87)	0.39	80.92%	0.00	REM
Metastasis	2	400	168	42.00%	1.67 (0.94–3.00)	0.08	34.94%	0.22	FEM
TNM staging	2	414	166	40.10%	0.98 (0.08–11.41)	0.99	95.72%	0.00	REM
Tumor nodularity	3	603	287	47.60%	0.67 (0.44–1.01)	0.054	0.00%	0.72	FEM
Tumor size	4	687	318	46.29%	1.19 (0.87–1.64)	0.28	0.00%	0.41	FEM

AFP, alpha-fetoprotein; CI, confidence interval; FEM, fixed-effects model; FOXO3, forkhead box O3; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HR, hazard ratio; OR, odds ratio; OS, overall survival; REM, random-effects model; TNM, tumor-node-metastasis.

♦ 2.4. Subgroup Analysis for OS and Invasion

In order to find the heterogeneity sources for every heterogeneous variable analyzed, subgroup analysis was carried out for parameters involving more than two original studies according to sample size, NOS score, patients' origin or follow-up time.

Regarding OS parameter, heterogeneity was successfully resolved when NOS ≤ 6 or follow-up > 60 months (I^2 = 15.43% and Q-test p = 0.28), also finding a significant correlation between high FOXO3 levels and poor OS **(Figure 3a, Table 2)**. Moreover, heterogeneity in invasion was solved in the subgroup NOS = 6 and Korean provenance (I^2 = 0.00% and Q-test p = 0.39), observing also for the first time a significant correlation with high levels of FOXO3 (OR, 2.95; 95% CI, 1.67–5.21; p = 0.00). Additionally, the elimination of Chen et al.[19] also led to an assumable heterogeneity and a significant association between FOXO3 overexpression and high probability of invasion **(Figure 3b, Table 2)**.


(a)
Subgroups by sample size (n)
OS
HR with 95% CI
Weight (%)
n > 100
Chen et al. 2017 2.90 [1.54, 5.47] 16.63
Ahn et al. 2018 1.33 [0.89, 1.98] 20.21
Song et al. 2020 3.62 [2.13, 6.14] 18.26
Zhou et al. 2020 1.76 [1.29, 2.40] 21.40
Heterogeneity: τ² = 0.15, I² = 74.73%, H² = 3.96 2.13 [1.37, 3.33]
Test of θi = θj: Q(3) = 10.71, p = 0.01
Test of θ = 0: z = 3.34, p = 0.00
n ≤ 100
Lu et al. 2009 0.97 [0.95, 0.99] 23.51
Heterogeneity: τ² = 0.00, I² = .%, H² = . 0.97 [0.95, 0.99]
Test of θi = θj: Q(0) = -0.00, p = .
Overall 1.79 [1.11, 2.89]
Heterogeneity: τ² = 0.25, I² = 91.66%, H² = 11.99
Test of θi = θj: Q(4) = 51.57, p = 0.00
Test of group differences: Qb(1) = 12.04, p = 0.00
Random-effects REML model
Subgroups by sample size (n)
OS
HR with 95% CI
Weight (%)
n > 200/300
Song et al. 2020 3.62 [2.13, 6.14] 18.26
Zhou et al. 2020 1.76 [1.29, 2.40] 21.40
Heterogeneity: τ² = 0.21, I² = 81.20%, H² = 5.32 2.44 [1.21, 4.94]
Test of θi = θj: Q(1) = 5.32, p = 0.02
Test of θ = 0: z = 2.49, p = 0.01
n ≤ 200/300
Lu et al. 2009 0.97 [0.95, 0.99] 23.51
Chen et al. 2017 2.90 [1.54, 5.47] 16.63
Ahn et al. 2018 1.33 [0.89, 1.98] 20.21
Heterogeneity: τ² = 0.24, I² = 88.89%, H² = 9.00 1.45 [0.80, 2.65]
Test of θi = θj: Q(2) = 13.84, p = 0.00
Test of θ = 0: z = 1.22, p = 0.22
Overall 1.79 [1.11, 2.89]
Heterogeneity: τ² = 0.25, I² = 91.66%, H² = 11.99
Test of θi = θj: Q(4) = 51.57, p = 0.00
Test of group differences: Qb(1) = 1.21, p = 0.27
Random-effects REML model
Subgroups by NOS score
OS
HR with 95% CI
Weight (%)
NOS > 5
Lu et al. 2009 0.97 [0.95, 0.99] 23.51
Chen et al 2017 2.90 [1.54, 5.47] 16.63
Ahn et al 2018 1.33 [0.89, 1.98] 20.21
Song et al 2020 3.62 [2.13, 6.14] 18.26
Heterogeneity: τ² = 0.36, I² = 91.83%, H² = 12.24 1.83 [0.98, 3.42]
Test of θi = θj: Q(3) = 37.57, p = 0.00
Test of θ = 0: z = 1.90, p = 0.06
NOS ≤ 5
Zhou et al 2020 1.76 [1.29, 2.40] 21.40
Heterogeneity: τ² = 0.00, I² = .%, H² = . 1.76 [1.29, 2.40]
Test of θi = θj: Q(0) = 0.00, p = .
Overall 1.79 [1.11, 2.89]
Heterogeneity: τ² = 0.25, I² = 91.66%, H² = 11.99
Test of θi = θj: Q(4) = 51.57, p = 0.00
Test of group differences: Qb(1) = 0.01, p = 0.91
Random-effects REML model
Subgroups by NOS score or follow-up (months)
OS
HR with 95% CI
Weight (%)
NOS > 6 / Follow-up ≤ 60
Lu et al. 2009 0.97 [0.95, 0.99] 23.51
Chen et al. 2017 2.90 [1.54, 5.47] 16.63
Song et al. 2020 3.62 [2.13, 6.14] 18.26
Heterogeneity: τ² = 0.50, I² = 92.03%, H² = 12.55 2.08 [0.90, 4.81]
Test of θi = θj: Q(2) = 35.21, p = 0.00
Test of θ = 0: z = 1.70, p = 0.09
NOS ≤ 6 / Follow-up > 60
Ahn et al. 2018 1.33 [0.89, 1.98] 20.21
Zhou et al. 2020 1.76 [1.29, 2.40] 21.40
Heterogeneity: τ² = 0.01, I² = 15.43%, H² = 1.18 1.58 [1.24, 2.02]
Test of θi = θj: Q(1) = 1.18, p = 0.28
Test of θ = 0: z = 3.68, p = 0.00
Overall 1.79 [1.11, 2.89]
Heterogeneity: τ² = 0.25, I² = 91.66%, H² = 11.99
Test of θi = θj: Q(4) = 51.57, p = 0.00
Test of group differences: Qb(1) = 0.38, p = 0.54
Random-effects REML model for NOS > 6 / Follow-up ≤ 60
Fixed-effects inverse-variance model for NOS ≤ 6 / Follow-up > 60
Subgroups by follow-up (months)
OS
HR with 95% CI
Weight (%)
Follow-up > 120/240
Ahn et al. 2018 1.33 [0.89, 1.98] 20.21
Heterogeneity: τ² = 0.00, I² = .%, H² = . 1.33 [0.89, 1.98]
Test of θi = θj: Q(0) = 0.00, p = .
Follow-up ≤ 120/240
Lu et al. 2009 0.97 [0.95, 0.99] 23.51
Chen et al. 2017 2.90 [1.54, 5.47] 16.63
Song et al. 2020 3.62 [2.13, 6.14] 18.26
Zhou et al. 2020 1.76 [1.29, 2.40] 21.40
Heterogeneity: τ² = 0.32, I² = 93.12%, H² = 14.54 1.96 [1.08, 3.55]
Test of θi = θj: Q(3) = 49.24, p = 0.00
Test of θ = 0: z = 2.21, p = 0.03
Overall 1.79 [1.11, 2.89]
Heterogeneity: τ² = 0.25, I² = 91.66%, H² = 11.99
Test of θi = θj: Q(4) = 51.57, p = 0.00
Test of group differences: Qb(1) = 1.12, p = 0.29
Random-effects REML model
(b)
Subgroups by sample size (n)
Invasion
Odds Ratio with 95% CI
Weight (%)
n > 200
Song et al. 2020 1.59 [0.93, 2.73] 25.55
Heterogeneity: τ² = 0.00, I² = .%, H² = . 1.59 [0.93, 2.73]
Test of θi = θj: Q(0) = 0.00, p = .
n ≤ 200
Chen et al. 2017 0.31 [0.12, 0.78] 22.00
Ahn et al. 2018 3.65 [1.83, 7.28] 24.27
Ahn et al. 2018 1.60 [0.55, 4.65] 20.54
Ahn et al. 2018 6.28 [0.34, 115.34] 7.63
Heterogeneity: τ² = 1.29, I² = 81.85%, H² = 5.51 1.52 [0.42, 5.56]
Test of θi = θj: Q(3) = 18.58, p = 0.00
Test of θ = 0: z = 0.63, p = 0.53
Overall 1.51 [0.59, 3.87]
Heterogeneity: τ² = 0.83, I² = 80.92%, H² = 5.24
Test of θi = θj: Q(4) = 18.58, p = 0.00
Test of group differences: Qb(1) = 0.00, p = 0.95
Random-effects REML model
Subgroups by NOS score or patients' origin
Invasion
Odds Ratio with 95% CI
Weight (%)
NOS = 6 / Korea
Ahn et al. 2018 3.65 [1.83, 7.28] 24.27
Ahn et al. 2018 1.60 [0.55, 4.65] 20.54
Ahn et al. 2018 6.28 [0.34, 115.34] 7.63
Heterogeneity: I² = 0.00%, H² = 0.95 2.95 [1.67, 5.21]
Test of θi = θj: Q(2) = 1.90, p = 0.39
Test of θ = 0: z = 3.73, p = 0.00
NOS = 7 / China
Chen et al. 2017 0.31 [0.12, 0.78] 22.00
Song et al. 2020 1.59 [0.93, 2.73] 25.55
Heterogeneity: τ² = 1.20, I² = 88.95%, H² = 9.05 0.73 [0.15, 3.65]
Test of θi = θj: Q(1) = 9.05, p = 0.00
Test of θ = 0: z = -0.38, p = 0.70
Overall 1.51 [0.59, 3.87]
Heterogeneity: τ² = 0.83, I² = 80.92%, H² = 5.24
Test of θi = θj: Q(4) = 18.58, p = 0.00
Test of group differences: Qb(1) = 2.35, p = 0.13
Fixed-effects inverse-variance model for NOS = 6 / Korea
Random-effects REML model for NOS = 7 / China
Subgroup removing Chen et al. 2017
Invasion
Odds Ratio with 95% CI
Weight (%)

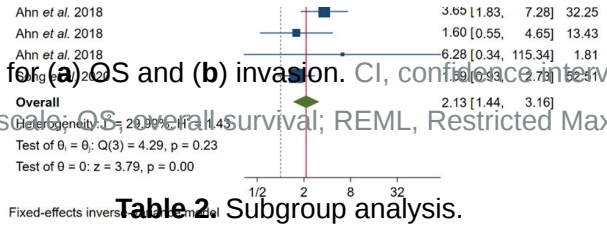


Figure 3. Subgroup analysis for (**a**) OS and (**b**) invasion. CI, confidence interval; HR, hazard ratio; NOS, Newcastle–Ottawa scale; OS, overall survival; REML, Restricted Maximum Likelihood.

Table 2. Subgroup analysis.

Subgroups	Number of Studies (n)	Number of Cases (n)	Samples with High FOXO3 Expression (n)	High FOXO3 Expression (%)	Pooled OR or HR		Test for Heterogeneity		Model Used
					95% CI	*p*-Value	I^2	Q-Test *p*-Value	
OS									
Sample size (n)									
n > 100	4	968	492	50.83%	2.13 (1.37–3.33)	0.00	74.73%	0.01	REM
n ≤ 100	1	74	37	50.00%	0.97 (0.95–0.99)	-	-	-	-
n > 200/300	2	679	329	48.45%	2.44 (1.21–4.94)	0.01	81.20%	0.02	REM
n ≤ 200/300	3	363	200	55.10%	1.45 (0.80–	0.22	88.89%	0.00	REM

					2.65)				
NOS score (threshold 5)									
NOS > 5	4	677	438	64.70%	1.83 (0.98–3.42)	0.06	91.83%	0.00	REM
NOS ≤ 5	1	365	91	24.93%	1.76 (1.29–2.40)	-	-	-	-
NOS score (threshold 6)									
NOS > 6	3	490	317	64.69%	2.08 (0.90–4.81)	0.09	92.03%	0.00	REM
NOS ≤ 6	2	552	212	38.41%	1.58 (1.24–2.02)	0.00	15.43%	0.28	FEM
Follow-up (months)									
>60	2	552	212	38.41%	1.58 (1.24–2.02)	0.00	15.43%	0.28	FEM
≤60	3	490	317	64.69%	2.08 (0.90–4.81)	0.09	92.03%	0.00	REM

>120/240	1	187	121	64.71%	1.33 (0.89–1.98)	-	-	-	-
≤120/240	4	855	408	47.72%	1.96 (1.08–3.55)	0.03	93.12%	0.00	REM
Invasion									
Sample size (n)									
n > 200	1	227	92	40.53%	1.59 (0.93–2.73)	-	-	-	-
n ≤ 200	4	663	405	61.09%	1.52 (0.42–5.56)	0.53	81.85%	0.00	REM
NOS score									
NOS = 6	3	561	363	64.71%	2.95 (1.67–5.21)	0.00	0.00%	0.39	FEM
NOS = 7	2	329	134	40.73%	0.73 (0.15–3.65)	0.70	88.95%	0.00	REM
Patients' origin									

China	2	329	134	40.73%	0.73 (0.15–3.65)	0.70	88.95%	0.00	REM
Korea	3	561	363	64.71%	2.95 (1.67–5.21)	0.00	0.00%	0.39	FEM
Without Chen et al.[19]									
	4	788	455	57.74%	2.13 (1.44–3.16)	0.00	29.99%	0.23	FEM

CI, confidence interval; FEM, fixed-effects model; FOXO3, forkhead box O3; HR, hazard ratio; NOS, Newcastle–Ottawa scale; OR, odds ratio; OS, overall survival; REM, random-effects model.

3. Discussion

Asymptomatic presentation at early stages, deficient diagnostic techniques and post-therapy recurrence are common features of HCC, a lethal primary liver tumor with disappointing prognosis[2][6][15][16][19][25]. Even though increasing efforts are being put into biomarker discovery[24][25][26][27], effective molecules able to improve HCC detection and predict therapy response are still lacking. Meanwhile, it has been suggested that FOXO3 deregulation could be involved in cancer emergence[10][12][16] and progression[12][16][19], but the exact linkage between FOXO3 expression and primary liver cancer has not been clarified yet. Therefore, the current study was carried out to accurately determine the relationship of FOXO3 high expression with tumor development, survival rate and clinicopathological features, examining the potential usefulness of this factor as a diagnostic and prognostic biomarker for HCC monitoring.

This systematic review with meta-analysis, mainly accomplished with Chinese population, which is not surprising since most new HCC cases usually come from China[28], detected a significant correlation between FOXO3 high expression and HCC pathogenesis. Interestingly, Lu et al.[29] evidenced that enhanced FOXO3 expression and activity is associated with strong liver damage and overexpression of HCC-related genes. Additional reports from pre-clinical studies also indicated that FOXO3 upregulation is related to HCC oncogenicity[17][30][13]. Contrariwise, Wu et al.[31] described that reduction in FOXO3 nuclear translocation and activity could be involved in sepiapterin reductase-mediated HCC progression. Thus, this meta-analysis supports the findings reported by the majority of

studies and suggests that upregulation of FOXO3 may constitute a suitable diagnostic factor able to complement classic techniques.

Furthermore, a significant association between FOXO3 overexpression and poor survival outcomes was registered in HCC patients, indicating that FOXO3 could constitute a negative prognostic factor in this tumor. Similar reports were shown in invasive ductal breast carcinoma[32], glioblastoma[33] and triple-negative breast cancer (TNBC) samples[34]. Otherwise, Zhao et al.[35] observed that FOXO3 downregulation could be linked with the enhancement of cell proliferation promoted by thyroid hormone receptor-interacting protein 6 (TRIP6). However, correlation between TRIP6 and FOXO3 expression in HCC individuals and its impact on survival rate were not assessed[35].

Additionally, it has been found that high FOXO3 levels may trigger HCC invasiveness. Different articles also determined that FOXO3 expression accentuates invasiveness and tumor expansion in glioblastoma[33], pancreatic cancer[36] and HeLa and melanoma MDA-MB-435 cells[37], being also correlated to perineural invasion in TNBC samples[34]. Contrariwise, FOXO3 oppositely impacted the invasive capabilities of breast tumors depending on the estrogen receptor α (ERα) status[38]. Moreover, Yang et al.[39] demonstrated the potential of bortezomib to inhibit cell migration and invasion by upregulating FOXO3 in cholangiocarcinoma and HCC models. However, these results were not tested in HCC patients, and bortezomib does not represent a major chemotherapeutic drug within the HCC field.

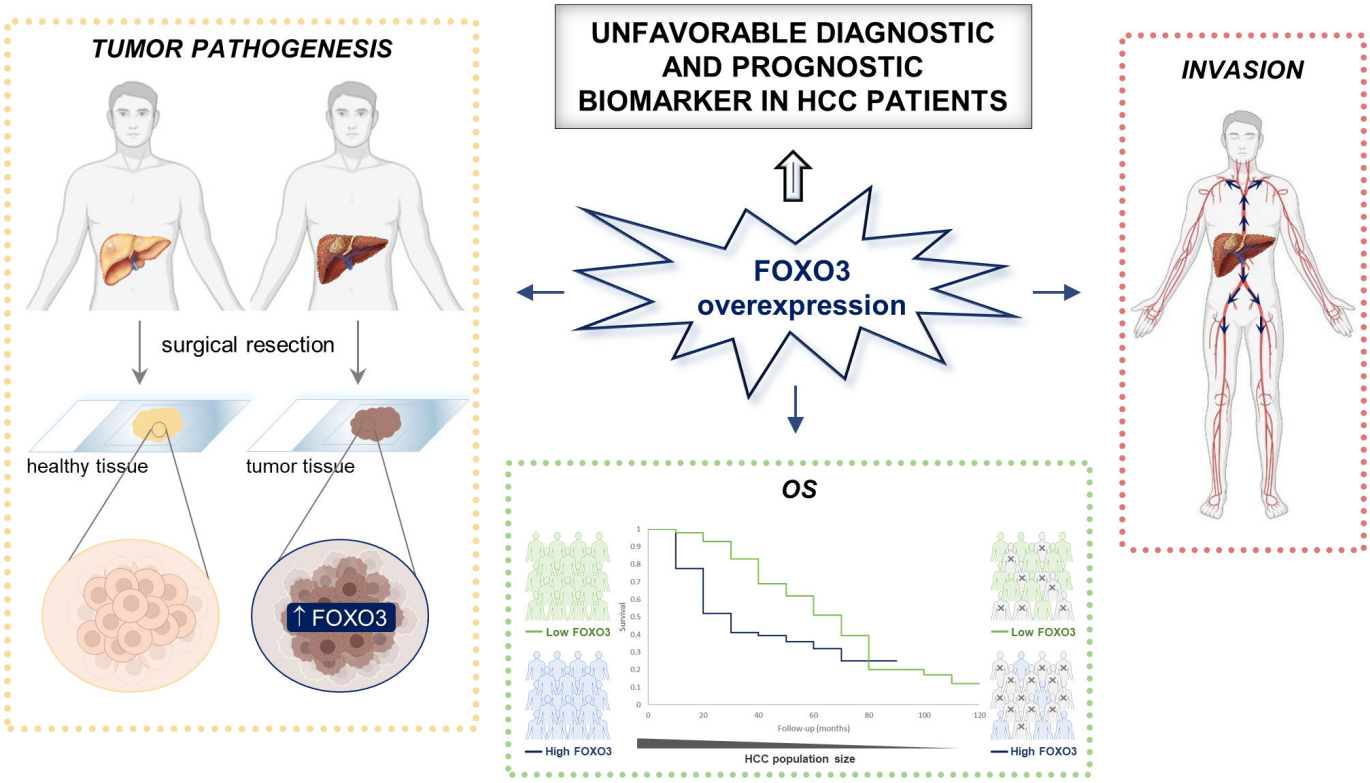
On the other hand, although no correlation was found between FOXO3 levels and any other evaluated clinicopathological feature, Chen et al.[40] indicated that FOXO3 could participate in the HBV-mediated HCC tumorigenesis. With regard to studies accomplished in other tumors, an investigation conducted with nasopharyngeal carcinoma samples observed that low FOXO3 expression correlates with advanced clinical stages and higher T stages, as well as with lymph node metastasis and distant metastasis[41]. Reduced FOXO3 levels in colorectal cancer[42], esophageal squamous cell carcinoma (ESCC)[43] and pancreatic ductal adenocarcinoma samples[44] have been also associated with more advanced disease. Deregulation of FOXO3 levels has shown to differentially influence lymph node metastasis in invasive ductal carcinoma[32], TNBC[34] and bladder carcinoma[45], finding that the interplay β-catenin-FOXO3 can be a metastasis promoter in colon cancer[46]. Besides, FOXO3 downregulation in ESCC patients accounted for lymph node metastasis[43], and its low expression correlated with a larger tumor size in gastric adenocarcinoma[47].

Altogether, these reports highlight the double-edged action played by FOXO3, finding that deregulation of FOXO3 expression and activity may lead to cancer promotion or suppression depending on the cancer type, cellular context or genomic profile.

4. Conclusions

In conclusion, this study proved for the first time that an enhanced FOXO3 expression could be an unfavorable clinical factor with diagnostic and prognostic significance in HCC, being associated with tumor development, poor OS and high risk of invasion. Therefore, the evaluation of FOXO3 levels could constitute a promising approach to

optimize and complement HCC detection and, specifically, to guide patient surveillance and make an accurate prognosis.



Graphical Abstract. FOXO3, Forkhead box O3; HCC, hepatocellular carcinoma; OS, overall survival.

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