

Research Article

Clinical and Survival Impact of Sex-Determining Region Y-Box 2 in Colorectal Cancer: An Integrated Analysis of the Immunohistochemical Study and Bioinformatics Analysis

Kun Song,¹ Jingduo Hao,¹ Zuyin Ge,¹ and Pushi Chen ²

¹Department of General Surgery, People's Hospital of Zhenhai, Ningbo, Zhejiang 315202, China

²Health Management Center, Ningbo First Hospital, Ningbo, Zhejiang 315000, China

Correspondence should be addressed to Pushi Chen; 314287075@qq.com

Received 14 August 2019; Revised 30 November 2019; Accepted 30 December 2019; Published 13 February 2020

Academic Editor: Rossana Berardi

Copyright © 2020 Kun Song et al. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Transcription factor sex-determining region Y-box 2 (SOX2) involves in the maintenance of cancer stem cells. However, the role of SOX2 in colorectal cancer (CRC) remains unclear. This study was conducted to investigate the effect of SOX2 on CRC. Studies were searched using electronic databases. The combined odds ratios (ORs) or hazard ratios (HRs: multivariate Cox survival analysis) with their 95% confidence intervals (CIs) were calculated. The Cancer Genome Atlas (TCGA) and GEO datasets were further applied to validate the survival effect. The functional analysis of SOX2 was investigated. In this work, 13 studies including 2337 patients were identified, and validation data were enrolled from TCGA and GEO datasets. SOX2 expression was not significantly related to age, gender, microsatellite instability (MSI) status, clinical stage, histological grade, tumor size, pT-stage, lymph node metastasis, distal metastasis, and cancer-specific survival (CSS) but was correlated with worse overall survival (OS: $n = 536$ patients) ($P < 0.05$). Furthermore, TCGA data demonstrated similar results, with no significant correlation between SOX2 and pathological characteristics. Further validation data (OS: $n = 1408$ and disease-free survival (DFS): $n = 1367$) showed that SOX2 expression was correlated with worse OS (HR = 1.35, 95% CI: 1.11–1.65, $P = 0.004$) and DFS (HR = 1.30, 95% CI: 1.04–1.62, $P = 0.02$). The functional analyses showed that SOX2 involved in cell-cell junction, focal adhesion, extracellular matrix- (ECM-) receptor interaction, and MAP kinase activity. Our findings suggest that SOX2 expression may be correlated with the worse prognosis of CRC.

1. Introduction

Colorectal cancer (CRC) is one of the most common malignant tumors and a major cause of cancer-related death in the world and in China [1, 2]. According to the GLOBOCAN estimates, approximately 1,800,977 new cases were diagnosed with CRC, leading to approximately 861,663 deaths due to this disease in 2018 worldwide [1]. Although some significant achievements have been made in early detection and treatment in recent years, most patients are diagnosed with advanced disease and show a poor 5-year survival rate [3–5]. Therefore, it is needed to find new efficient biomarkers for the treatment of CRC along with the prognosis.

Cancer stem cells (CSCs) are a special subpopulation of tumor cells and have the ability and characteristics of self-

renewal and multilineage differentiation and proliferation potential, which are related to tumor progression, metastasis, recurrence, prognosis, and drug resistance [6–8]. Many CSC-related markers have been identified and reported to be associated with poor prognosis and resistance to therapy in cancer [9, 10]. Sex-determining region Y-box 2 (SOX2), a high mobility group (HMG) DNA-binding domain, is mapped to human chromosome 3q26.3–q27 and belongs to a key transcription factor [11]. SOX2 regulates the self-renewal and pluripotency of undifferentiated stem cells such as human embryonic stem cells and plays an important role in maintaining the stem cell-like features in cancer cells [12–14]. Additionally, SOX2 involves in the migration, invasion, and proliferation of cancer cells and resistance to therapy [15, 16]. SOX2 has been reported to be commonly

expressed in many human cancers, such as breast cancer, lung cancer, esophageal cancer, and CRC [17]. For example, SOX2 expression is correlated with worse prognosis in breast cancer and gastric cancer [18, 19]. While SOX2 expression shows a favorable prognosis in non-small cell lung cancer and cervical cancer [20, 21]. Therefore, it is of great importance to determine the prognostic role of SOX2 expression in CRC.

The previous meta-analysis only included eight studies with small sample sizes ($n=1113$) and only analyzed the correlation between SOX2 and overall survival (OS) and a small part of the clinical features of CRC. Additionally, the result on OS was not reasonable [22]. Therefore, the significance of SOX2 expression in CRC is not fully understood. For example, Lundberg et al. reported that SOX2 expression was not related to cancer-specific survival in CRC [23], but SOX2 expression was correlated with worse cancer-specific survival by Miller et al. [24]. Thus, the current work with 3745 CRCs was performed to determine the survival impact of SOX2 expression on CRC ((cancer-specific survival (CSS), overall survival (OS), and disease-free survival (DFS)) and to evaluate the association between SOX2 expression and general clinicopathological characteristics.

2. Materials and Methods

2.1. Literature Search. This meta-analysis was performed based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement [25] (Supplementary Materials). The electronic databases PubMed, EMBASE, and Web of Science were comprehensively searched to identify eligible publications until July 29, 2019, by searching the following key words and search terms: “colorectal cancer OR colorectal tumor OR colorectal carcinoma OR colorectal neoplasm OR CRC OR rectal cancer OR rectal tumor OR rectal carcinoma OR colon cancer OR colon tumor OR colon carcinoma” and “SOX2 OR SOX-2 OR Sex-determining region Y-box protein 2 OR Sex-determining region Y box-2 OR Sex-determining region Y-box 2 OR SRY box-2” (Supplementary Materials). Moreover, the reference lists of the identified articles were also examined to find other relevant studies.

2.2. Eligibility Criteria. For the enrollment of publications, the main inclusion criteria were included as follows: (1) the patients with CRC were reported; (2) studies reported the detection of SOX2 using immunohistochemistry (IHC); (3) expression status of SOX2 was defined from the original articles; (4) studies provided available data to assess the correlation between SOX2 expression and clinicopathological parameters; (5) studies provided sufficient hazard ratio (HR) with 95% confidence interval (CI) to evaluate the prognostic impact of SOX2 expression on CRC patients based on multivariate Cox survival analysis. If the results of interest were not completely reported, the corresponding author will be contacted via e-mail. Only the recent article or the article with the most complete data was selected when multiple articles using overlapping tissue samples from the

same institute were published. We mainly excluded review articles, letters, conference abstracts, case reports, cell line/animal studies, and articles lacking sufficient data.

2.3. Data Extraction and Study Quality Assessment. The relevant data and search of the included studies were conducted from two independent authors (KS and JH) using standardized forms, including the surname of the first author, time of publication, country, ethnicity, median/mean age, disease stage, antibody information, number of participants, cutoff values of SOX2, expression frequency, clinicopathological parameters such as age, gender, microsatellite instability (MSI) status, clinical stage, histological grade, tumor size, pT-stage, lymph node metastasis, and distal metastasis, and the survival data of multivariate Cox analysis such as cancer-specific survival (CSS), overall survival (OS), and disease-free survival (DFS). The quality of the available publications was assessed according to the Newcastle–Ottawa Scale (NOS) for cohort studies [26, 27]. Three parameters of quality included a total of nine scores: selection (0–4), comparability (0–2), and outcome assessment (0–3). In this meta-analysis, the publication with ≥ 6 scores was considered to be of high quality, and the NOS score with < 6 was defined as low-quality study. Any disagreements in the selected literature were discussed by all authors and then reached a consensus.

2.4. Validation Data from TCGA. The RNA-sequencing data and corresponding clinical information on CRC were obtained from The Cancer Genome Atlas (TCGA) (<https://portal.gdc.cancer.gov/repository>). Eventually, 618 cases with CRC with sufficient expression data and clinical information were selected.

2.5. Functional Analysis of SOX2. Association between SOX2 and genes was analyzed using TCGA sequencing data. Spearman coefficients with an absolute value of > 0.2 and $P < 0.001$ were applied for SOX2. The potential function and biological mechanism of the SOX2 gene such as GO (Gene Ontology) and KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways were investigated by clusterProfiler package.

2.6. Survival Analysis from Validation Datasets. Normalized GSE17538 ($n=232$ patients), GSE39582 ($n=558$ patients), and TCGA ($n=618$ patients) datasets were applied due to sufficient survival information and the batch effects were adjusted using the ComBat method [28, 29]. Finally, 1408 CRC patients were used to further confirm whether SOX2 expression was still correlated with worse OS and 1367 CRC patients were used to further validate whether SOX2 expression was still related to disease-free survival (DFS). The optimal cutoff value (SOX2: 3.31) was selected and survival analysis was performed using “survival and survminer” packages.

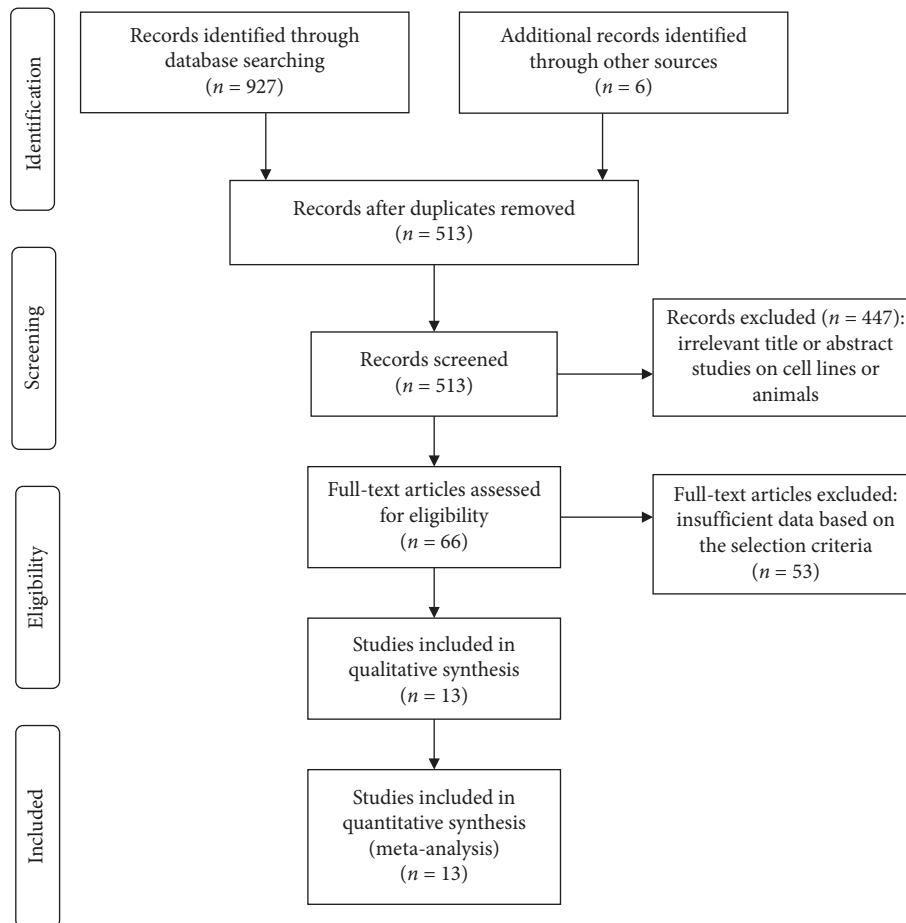


FIGURE 1: Flow diagram of the study selection process.

2.7. Statistical Analysis. Data on meta-analysis were obtained from the original articles. The pooled odds ratios (ORs) and the corresponding 95% CIs were calculated to assess the relationship of SOX2 expression with the clinicopathological parameters. The pooled HRs with their 95% CIs were performed to estimate the prognostic impact of SOX2 expression on CRC patients using multivariate Cox survival analysis. As described in the report of IntHout et al., the Hartung–Knapp–Sidik–Jonkman (HKSJ) method was applied to improve the reliability of the pooled results with ≤ 10 studies in the current meta-analysis [30]. The heterogeneity assumption between studies was measured using Cochran’s Q statistic [31]. A Q test of $P < 0.1$ was considered to be a significant heterogeneity. When substantial heterogeneity was detected, sensitivity analyses were conducted to estimate the change of heterogeneity and stability of an individual study on the recalculated results by removing a single study [32]. The possible publication bias was performed by Egger’s test [33]. The pooled ORs and HRs were calculated using “metafor” package via R version 3.5.1 [R Core Team, 2018]. Results of publication bias were carried out using Stata software (version 12.0, Stata Corporation, College Station, TX, US). The code for the relevant examples is listed in Supplementary Materials.

For bioinformatics validation data, the TCGA patients were divided into positive and negative groups based on the median value of SOX2 expression. The relationships between SOX2 expression and the clinicopathological parameters were performed using the univariate logistic regression analysis. The clinicopathological parameters included age, MSI status, clinical stage, lymph node metastasis, distal metastasis, venous invasion, and lymphatic invasion. Analysis for validation data was performed by using R version 3.5.1 [R Core Team, 2018].

3. Results

3.1. Study Characteristics. The flow diagram for the study selection is presented in Figure 1. Based on the selection criteria, inappropriate studies were excluded. Finally, 13 studies published between 2010 and 2019 were identified in the present meta-analysis [6, 14, 23, 24, 34–42], including 2337 patients with CRC. Eligible studies were conducted in China, Korea, Singapore, Australia, Germany, Sweden, and The Netherlands. Among these studies, expression of SOX2 was evaluated by IHC. 10 studies with 1431 cases evaluated the relationship between SOX2 expression and the clinicopathological parameters [14, 23, 24, 35–41]. Five studies

TABLE 1: Main characteristics of the eligible publications.

First author	Country	Age	Stage	Antibody	Sources of antibody	Staining	Cutoff values (IHC)	Cancer Total (E+ %)	Clinical features	Survival-MA
Ong et al. [42]	Singapore	NA	1-4	Anti-SOX2	Clone 57CT23.3.4; Abcam, Cambridge, MA	Cytoplasm	10%	310 (NA)	No	CSS
Neumann et al. [41]	Germany	66.5	NA	Anti-SOX2	Clone D6D9, cell Signaling technology, Danvers, MA	Nuclei	10%	114 (21.1%)	Yes	NA
Han et al. [40]	China	NA	NA	Anti-SOX2	Epitomics	NA	NA	44 (20.5%)	Yes	NA
Lee et al. [39]	Korea	NA	1-4	Anti-SOX2	Abcam, Cambridge, UK	Nuclei	5%	110 (17.3%)	Yes	NA
Liu et al. [43]	China	NA	1-4	Anti-SOX2	Cell Signaling Technology, Inc ab75485, Abcam, Cambridge, MA	Nuclei	≥ 2 scores	67 (22.4%)	Yes	NA
Ma et al. [38]	China	65	1-4	Anti-SOX2	Abcam, Cambridge, MA	NA	>1.40	89 (50.6%)	Yes	NA
Raghoebir et al. [37]	The Netherlands	NA	NA	Anti-SOX2	Immune systems	Nuclei	5%	135 (20.7%)	Yes	NA
Lundberg et al. [23]	Sweden	NA	1-4	Anti-SOX2	Abcam, Cambridge, UK	Nuclei	NA	441 (10.7%)	Yes	CSS
Yan et al. [36]	China	65	3-4	Anti-SOX2	Abcam	NA	2.5	280 (41.8%)	Yes	NA
Miller et al. [24]	Australia	66.7	3	Anti-SOX2	EPR3131; Abcam cell Signaling Technology, Danvers	Nuclei	NA	104 (18.3%)	Yes	CSS, OS
Zheng et al. [35]	China	NA	1-4	Anti-SOX2	Mass cat. no. 636675, EMD Millipore	NA	NA	47 (80.9%)	Yes	NA
Han et al. [44]	Korea	59.8	3	Anti-SOX2	Billerica, MA, USA	NA	≥ 1 score	164 (82.3%)	No	DFS
Li et al. [34]	China	NA	1-4	Anti-SOX2	#ab92494	NA	NA	432 (NA)	No	OS

NA: not applicable; IHC: immunohistochemistry; E+: positive/high expression; MA: multivariate Cox analysis; DFS: disease-free survival; OS: overall survival; CSS: cancer-specific survival.

TABLE 2: Summary of results from this meta-analysis.

Variables	OR [95% CI]	Heterogeneity (p)	t value	P value	Studies	Cases
Age (≥ 60 vs. < 60 years)	0.80 [0.13, 4.77]	0.618	-1.621	0.352	2	530
Gender (male vs. female)	0.91 [0.51, 1.64]	0.138	-0.439	0.684	5	991
MSI status (MSI vs. MSS)	1.40 [0.66, 3.00]	0.513	1.422	0.25	4	775
Clinical stage (stages 3-4 vs. 1-2)	1.34 [0.32, 5.64]	0.028	0.647	0.564	4	619
Histological grade (grades 3-4 vs. 1-2)	1.65 [0.62, 4.33]	0.004	1.428	0.227	5	999
Tumor size (≥ 5 vs. ≤ 5 cm)	0.78 [0.00, 1913.29]	0.018	-0.398	0.759	2	347
pT-stage (T 3-4 vs. 1-2)	1.12 [0.36, 3.43]	0.065	0.308	0.778	4	526
Lymph node metastasis (yes vs. no)	3.19 [0.58, 17.66]	0.368	2.916	0.1	3	225
Distal metastasis (yes vs. no)	1.26 [0.14, 10.91]	0.015	0.454	0.694	3	438

OR: odds ratio; CI: confidence interval; MSI: microsatellite instability; MSS: microsatellite stability.

with 1451 cases assessed the association between SOX2 expression and the prognosis using multivariate Cox survival analysis [6, 23, 24, 34, 42]. 13 studies were considered to be of high quality by using NOS. The details of the included studies are summarized in Table 1 and Table S1.

3.2. Correlation of SOX2 Expression with Clinicopathological Variables. A summary of the calculated results is shown in Table 2. No association was found between SOX2 expression and age (two studies with 530 cases, ≥ 60 vs. < 60 years:

OR = 0.80, $P = 0.352$), gender (five studies with 991 cases, male vs. female: OR = 0.91, $P = 0.684$), MSI status (four studies with 775 cases, MSI vs. microsatellite stability (MSS): OR = 1.40, $P = 0.25$) (Figure 2).

No correlation was found between SOX2 expression and clinical stage (four studies with 619 cases, stages 3-4 vs. 1-2: OR = 1.34, $P = 0.564$) and histological grade (five studies with 999 cases, grades 3-4 vs. 1-2: OR = 1.65, $P = 0.227$) (Figure 3).

No relationship was observed between SOX2 expression and tumor size (two studies with 347 cases, ≥ 5 vs. ≤ 5 cm: OR = 0.78, $P = 0.759$), pT-stage (four studies with 526 cases,

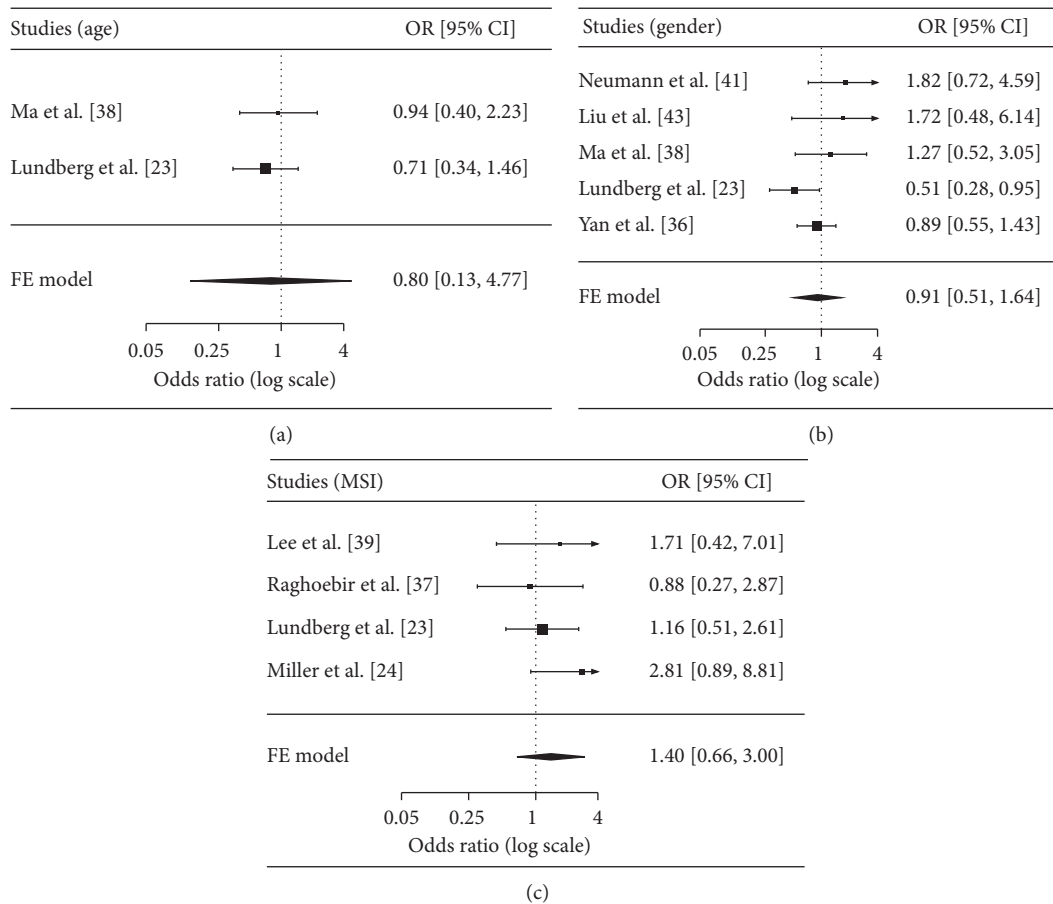


FIGURE 2: Forest plot for the correlation between SOX2 and age, gender, and MSI status. (a) Age (≥ 60 vs. < 60 years); (b) gender (male vs. female); (c) MSI status (MSI vs. MSS). OR: odds ratio; CI: confidence interval; MSS: microsatellite stability; MSI: microsatellite instability.

pT-stages 3-4 vs. 1-2: OR = 1.12, $P = 0.778$), lymph node metastasis (three studies with 225 cases, yes vs. no: OR = 3.19, $P = 0.1$), and distal metastasis (three studies with 438 cases, yes vs. no: OR = 1.26, $P = 0.694$) (Figure 3).

3.3. Survival Impact of SOX2 Expression Using Multivariate Cox Analysis. Data showed that SOX2 expression was not correlated with cancer-specific survival (CSS) of CRC in three studies with 855 patients (HR = 1.18, $P = 0.667$) (Figure 4) but was correlated with worse overall survival (OS) in two studies with 536 patients ($P < 0.05$) [24, 34].

3.4. Heterogeneity Analysis. Heterogeneity analysis was conducted between SOX2 expression and clinical stage, histological grade, and pT-stage. We conducted sensitivity analyses to estimate the change of the pooled results. When the study of Lundberg et al. [23] was deleted, the recalculated OR was still not correlated with the clinical stage (OR = 0.55, 95% CI = 0.06–4.66, $P = 0.350$), with no evidence of heterogeneity ($P = 0.272$). When the study of Han et al. [40] was omitted, the recalculated result was still not associated with the pT-stage (OR = 1.04, 95% CI = 0.30–3.54, $P = 0.908$), with no heterogeneity ($P = 0.216$). When the study of Yan et al. [36] was removed, the recalculated result

was significantly correlated with the histological grade (OR = 2.70, 95% CI = 1.15–6.37, $P = 0.035$), with no heterogeneity ($P = 0.250$).

3.5. Publication Bias. No publication bias was found between SOX2 expression and gender and histological grade ($P > 0.1$) (Figure S1).

3.6. TCGA. Six hundred eighteen cases with CRC were enrolled from TCGA. As shown in Table 3, the results using univariate logistic regression analysis showed that SOX2 expression was not significantly associated with age (≥ 60 vs. < 60 years: OR = 1.03, 95% CI = 0.73–1.46, $P = 0.859$), MSI status (MSI vs. MSS: OR = 1.08, 95% CI = 0.77–1.52, $P = 0.663$), clinical stage (stages 3-4 vs. 1-2: OR = 1.37, 95% CI = 0.99–1.89, $P = 0.059$), venous invasion (yes vs. no: OR = 1.12, 95% CI = 0.75–1.65, $P = 0.585$), lymphatic invasion (yes vs. no: OR = 1.27, 95% CI = 0.91–1.79, $P = 0.162$), lymph node metastasis (yes vs. no: OR = 1.37, 95% CI = 0.99–1.88, $P = 0.057$), and distal metastasis (yes vs. no: OR = 1.13, 95% CI = 0.72–1.79, $P = 0.597$), but SOX2 expression was correlated with gender (male vs. female: OR = 0.69, 95% CI = 0.5–0.94, $P = 0.02$).

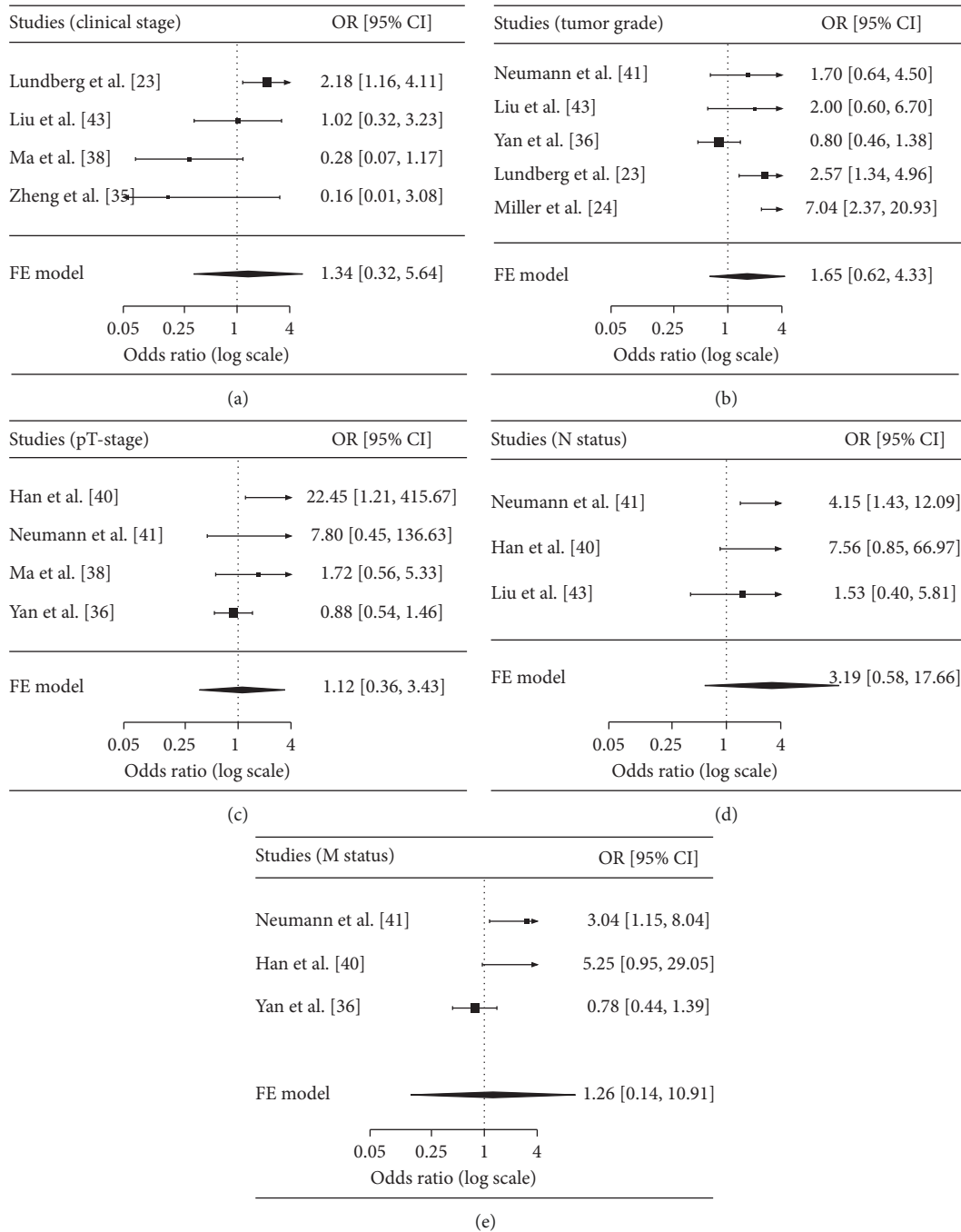


FIGURE 3: Forest plot for the correlation between SOX2 and clinical stage, histological grade, pT-stage, lymph node metastasis, and distal metastasis. (a) Clinical stage: stages 3-4 vs. 1-2; (b) tumor grade: grades 3-4 vs. 1-2; (c) pT-stage: T 3-4 vs. 1-2; (d) lymph node metastasis (N status): yes vs. no; (e) distal metastasis (M status): yes vs. no. OR: odds ratio; CI: confidence interval.

3.7. SOX2 Involves in Some Key Molecular Functions in CRC. GO and KEGG analyses showed that SOX2 involved in cell-cell junction, focal adhesion, extracellular matrix- (ECM-) receptor interaction, transmembrane receptor protein tyrosine kinase activity, transmembrane-ephrin receptor activity, semaphorin receptor activity, MAP kinase activity, mitogen-activated protein kinase binding, transmembrane receptor protein kinase activity, etc. in CRC (Figure 5), and

these pathways are closely associated with cancer development.

3.8. Further Survival Analysis from Validation Data. The validation data included 1408 patients with CRC, and the result also confirmed that SOX2 expression was significantly associated with worse OS (HR = 1.35, 95% CI: 1.11–1.65,

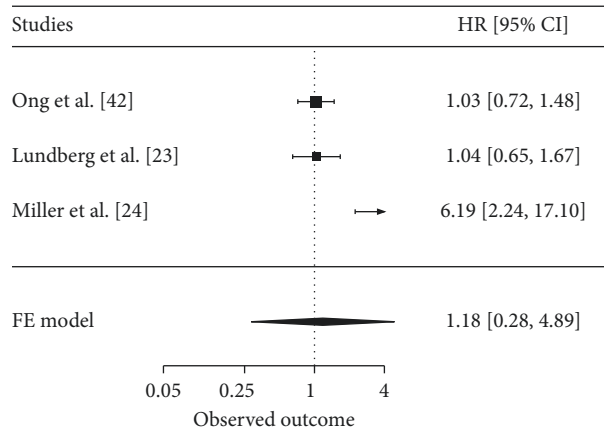


FIGURE 4: Forest plot for the correlation between SOX2 and cancer patients' prognosis using multivariate cox analysis in cancer-specific survival (CSS). HR: hazard ratio; CI: confidence interval.

TABLE 3: Correlation of SOX2 with the clinicopathological characteristics from the cancer genome atlas.

Variables	Comparison	OR with 95% CI	P	Cases
Age	≥60 vs. <60 years	1.03 (0.73–1.46)	0.859	618
Gender	Male vs. female	0.69 (0.5–0.94)	0.02	618
MSI status	MSI vs. MSS	1.08 (0.77–1.52)	0.663	618
Clinical stage	Stages 3-4 vs. 1-2	1.37 (0.99–1.89)	0.059	598
Venous invasion	Yes vs. no	1.12 (0.75–1.65)	0.585	536
Lymphatic invasion	Yes vs. no	1.27 (0.91–1.79)	0.162	557
Lymph node metastasis	Yes vs. no	1.37 (0.99–1.88)	0.057	615
Distal metastasis	Yes vs. no	1.13 (0.72–1.79)	0.597	545

OR: odds ratio; CI: confidence interval; MSI: microsatellite instability; MSS: microsatellite stability.

$P = 0.004$) (Figure 6(a)). Further validation data with 1367 CRC patients were used, and the result showed that SOX2 expression was associated with worse DFS (HR = 1.30, 95% CI: 1.04–1.62, $P = 0.02$) (Figure 6(b)).

4. Discussion

CSCs contribute to tumor metastasis and prognosis and therapeutic resistance [45, 46]. CSCs may offer new promising therapeutic targets of treatment modalities applicable to human various cancers [47, 48]. SOX2 involves in the development and maintenance of stem-like properties in cancer cells [14, 15]. SOX2 involves in many signaling pathways such as VEGF, MAPK, Notch, P53, Wnt, and Jak-STAT, regulates many expression of genes, and regulates self-renewal and differentiation of stem cells, which may contribute to migration, invasion, and proliferation of cancer cells and the stemness of cancer stem cells, thereby affecting cancer progression, prognosis, and resistance toward anticancer therapies [14, 15, 17]. SOX2 expression is found across a wide range of human cancers such as breast cancer, lung cancer, and esophageal cancer [17]. SOX2 expression is correlated with the prognosis of some human cancers such as non-small cell lung cancer and cervical cancer with better prognosis [20, 21] and breast cancer and gastric cancer with worse prognosis [18, 19]. Studies have reported that SOX2 is frequently expression in CRC [6, 34, 35]. However, the function of SOX2 and its survival

impact in patients with CRC are still largely uncertain. In the present work, we determined the clinicopathological effect of SOX2 and its expression on the prognostic impact of patients with CRC.

The relationships between SOX2 expression and the clinicopathological characteristics of CRC were investigated. The pooled data showed that SOX2 expression was not correlated with age and MSI status, which were consistent with the previous studies on age [23, 38] and MSI status [23, 24, 37, 39]. Further TCGA also showed no correlation with age and MSI status. Although no correlation was found between SOX2 expression and gender among all eligible studies, a large population with 441 cases reported that SOX2 expression was negatively correlated with gender and was lower in males than in females [23]. Further data from TCGA also demonstrated a negative association with gender, suggesting that more studies with larger sample sizes are needed to confirm this controversial finding. SOX2 expression was not associated with clinical stage, histological grade, tumor size, pT-stage, lymph node metastasis, and distal metastasis, which were in line with the previous publications regarding clinical stage [14, 35, 38], histological grade [14, 36, 41], tumor size [36], pT-stage [36, 38, 41], lymph node metastasis [14, 40], and distal metastasis [36]. Moreover, TCGA data also confirmed no significant association with clinical stage, lymph node metastasis, and distal metastasis. These results suggested that SOX2 expression was not significantly associated with progression and

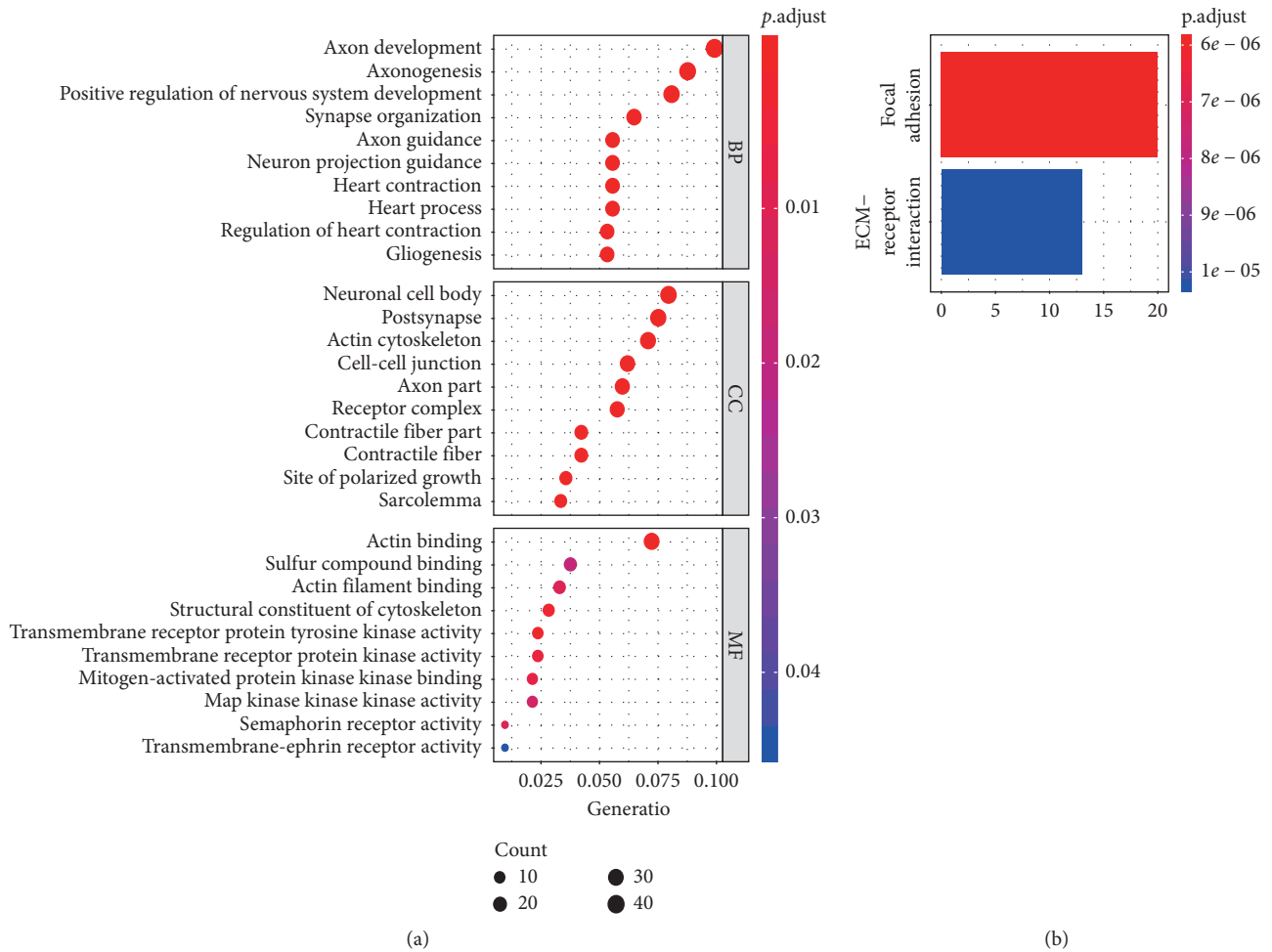


FIGURE 5: SOX2 involves in some cancer mechanisms, (a) GO (gene ontology) analysis; (b) KEGG (Kyoto Encyclopedia of Genes and Genomes) analysis.

metastasis of CRC. Further relevant studies are necessary to confirm our results in the future.

The survival impact of SOX2 expression in patients with CRC was performed based on multivariate Cox analysis. SOX2 expression was not correlated with CSS but was significantly associated with worse OS in CRC. Additionally, further validation data from TCGA and GEO datasets (OS: $n = 1408$ CRC patients and DFS: 1367 CRC patients) demonstrated that SOX2 expression was significantly correlated with worse OS (HR = 1.35, $P = 0.004$) and DFS (HR = 1.30, $P = 0.02$). These analyses suggested that SOX2 was an independent prognostic marker for predicting poor prognosis. Additionally, the functional analysis showed that SOX2 involved in cell-cell junction, focal adhesion, extra-cellular matrix- (ECM-) receptor interaction, transmembrane receptor protein tyrosine kinase activity, transmembrane-ephrin receptor activity, semaphorin receptor activity, mitogen-activated protein kinase binding, transmembrane receptor protein kinase activity, MAP kinase activity, etc., which further suggested that SOX2 may be closely linked with CRC prognosis.

There are several limitations in the present study. First, this meta-analysis has not been registered online. Second,

the main ethnic population was Asian and European; other ethnic groups, such as Africans, were lacking. Third, due to fewer studies, subgroups were insufficient and meta-regression analysis was not performed. However, heterogeneity analysis was carried out based on sensitivity analysis and the detailed reasons for the potential heterogeneity were not very certain. The possible use of the inappropriate and different conditions of IHC methods such as different/unclear cutoff values of SOX2 expression and different sources of anti-SOX2 antibody may lead to the potential sources of heterogeneity. For example, in the future, the expression of SOX2 is defined as positive or negative, which should be recommended using a uniform standard. Fourth, only Han et al. reported that SOX2 expression was correlated with DFS in 164 CRC cases (HR = 2.558, $P = 0.020$) [6]. Finally, sample sizes on the analyses between SOX2 expression and the clinicopathological features and the prognosis were relatively small in the meta-analysis, more studies with large study population are necessary to further determine whether SOX2 is correlated with clinical and prognostic value in CRC based on IHC methods.

In conclusion, the current study suggested that SOX2 expression was not significantly correlated with age, gender,

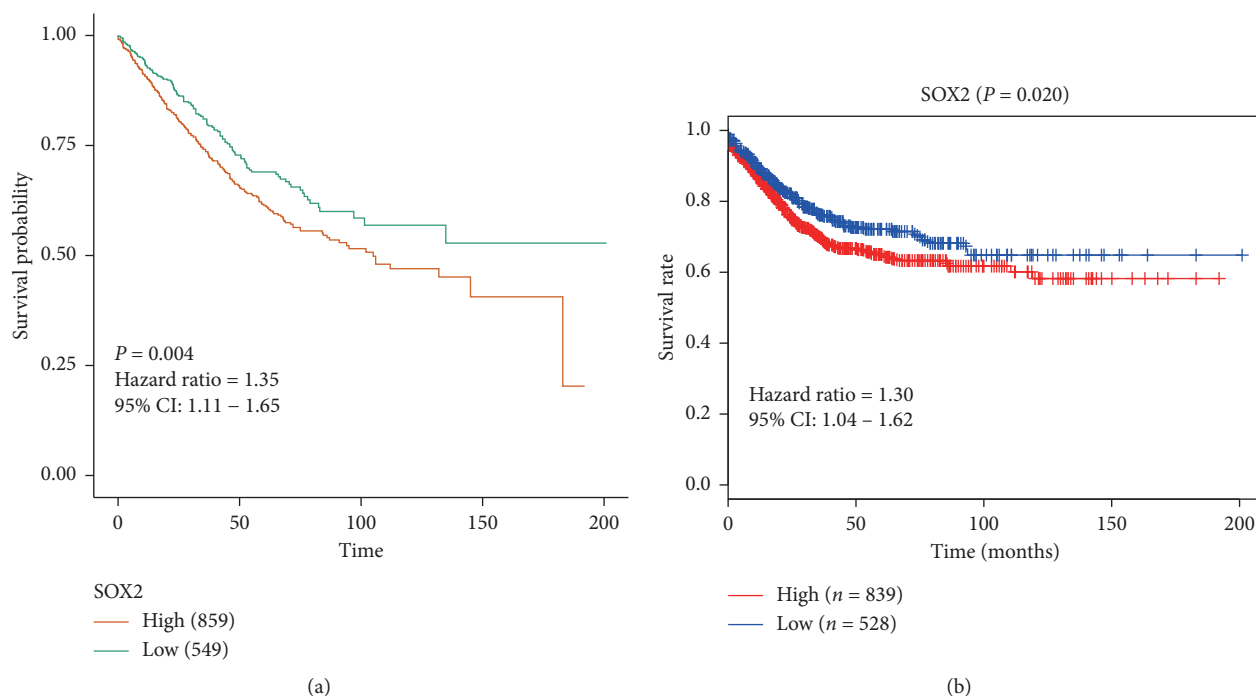


FIGURE 6: Survival analysis of SOX2 expression from validation data. (a) Overall survival (OS) in 1408 patients; (b) disease-free survival (DFS) in 1367 patients.

MSI status, clinical stage, histological grade, tumor size, pT-stage, lymph node metastasis, distal metastasis, and CSS but was associated with worse OS and DFS. Our data suggested that SOX2 may be used as an independent prognostic marker for worse prognosis in CRC. More large-scale prospective studies with larger sample sizes are required to further validate these findings.

Data Availability

Data are available in this manuscript.

Ethical Approval

All data collection and processing, including the consenting process, were performed after approval by all local institutional review boards and in accord with The Cancer Genome Atlas and Gene Expression Omnibus Human Subjects Protection and Data Access Policies.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Kun Song and Pushi Chen contributed to the study conception and design. Kun Song, Jingduo Hao, Zuyin Ge, and Pushi Chen contributed to drafting of the article and final approval of the submitted version. Kun Song, Jingduo Hao, Zuyin Ge, and Pushi Chen contributed to the analyses and interpretation of the data and completion of figures and tables. All authors read and approved the final manuscript.

Acknowledgments

The authors gratefully acknowledge The Cancer Genome Atlas and Gene Expression Omnibus.

Supplementary Materials

Table S1: data of the eligible publications with the clinico-pathological characteristics. Figure S1: publication bias using Egger's test. PRISMA 2009 Checklist Search terms Code for example. (*Supplementary Materials*)

References

- [1] F. Bray, J. Ferlay, I. Soerjomataram, R. L. Siegel, L. A. Torre, and A. Jemal, "Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries," *CA: A Cancer Journal for Clinicians*, vol. 68, no. 6, pp. 394–424, 2018.
- [2] W. Chen, R. Zheng, P. D. Baade et al., "Cancer statistics in China, 2015," *CA: A Cancer Journal for Clinicians*, vol. 66, no. 2, pp. 115–132, 2016.
- [3] M. H. Al Bandar and N. K. Kim, "Current status and future perspectives on treatment of liver metastasis in colorectal cancer," *Oncology Reports*, vol. 37, no. 5, pp. 2553–2564, 2017.
- [4] J. L. Diaz, S. M. Wanta, T. M. Fishbein, and A. Kroemer, "Developments in immunotherapy for gastrointestinal cancer," *Minerva Chirurgica*, vol. 70, no. 4, pp. 217–230, 2015.
- [5] H. Brenner, M. Kloor, and C. P. Pox, "Colorectal cancer," *The Lancet*, vol. 383, no. 9927, pp. 1490–1502, 2014.
- [6] S. H. Han, J. W. Kim, M. Kim et al., "Prognostic implication of ABC transporters and cancer stem cell markers in patients with stage III colon cancer receiving adjuvant FOLFOX-4

- chemotherapy,” *Oncology Letters*, vol. 17, no. 6, pp. 5572–5580, 2019.
- [7] Z. Pieterse, M. A. Amaya-Padilla, T. Singomat et al., “Ovarian cancer stem cells and their role in drug resistance,” *The International Journal of Biochemistry & Cell Biology*, vol. 106, pp. 117–126, 2019.
- [8] N. Yelle, D. Bakhshinyan, C. Venugopal, and S. K. Singh, “Introduction to brain tumor stem cells,” in *Methods in Molecular Biology*, vol. 1869, pp. 1–9, Springer, Berlin, Germany, 2019.
- [9] S. Han, T. Huang, X. Wu et al., “Prognostic value of ALDH1 and Nestin in advanced cancer: a systematic meta-analysis with trial sequential analysis,” *Therapeutic Advances in Medical Oncology*, vol. 11, Article ID 1758835919830831, 2019.
- [10] Z. Kozovska, V. Gabrisova, and L. Kucerova, “Colon cancer: cancer stem cells markers, drug resistance and treatment,” *Biomedicine & Pharmacotherapy*, vol. 68, no. 8, pp. 911–916, 2014.
- [11] L. Annovazzi, M. Mellai, V. Caldera, G. Valente, and D. Schiffer, “SOX2 expression and amplification in gliomas and glioma cell lines,” *Cancer Genomics Proteomics*, vol. 8, no. 3, pp. 139–147, 2011.
- [12] M.-Y. Chou, F.-W. Hu, C.-H. Yu, and C.-C. Yu, “SOX2 expression involvement in the oncogenicity and radiochemoresistance of oral cancer stem cells,” *Oral Oncology*, vol. 51, no. 1, pp. 31–39, 2015.
- [13] M. Herreros-Villanueva, J.-S. Zhang, A. Koenig et al., “SOX2 promotes dedifferentiation and imparts stem cell-like features to pancreatic cancer cells,” *Oncogenesis*, vol. 2, no. 8, p. e61, 2013.
- [14] H. Liu, L. Du, Z. Wen et al., “Sex determining region Y-box 2 inhibits the proliferation of colorectal adenocarcinoma cells through the mTOR signaling pathway,” *International Journal of Molecular Medicine*, vol. 32, no. 1, pp. 59–66, 2013.
- [15] L. Hüser, D. Novak, V. Umansky, P. Altevogt, and J. Utikal, “Targeting SOX2 in anticancer therapy,” *Expert Opinion on Therapeutic Targets*, vol. 22, no. 12, pp. 983–991, 2018.
- [16] P. Mu, Z. Zhang, M. Benelli et al., “SOX2 promotes lineage plasticity and antiandrogen resistance in TP53- and RB1-deficient prostate cancer,” *Science*, vol. 355, no. 6320, pp. 84–88, 2017.
- [17] E. L. Wuebben and A. Rizzino, “The dark side of SOX2: cancer—a comprehensive overview,” *Oncotarget*, vol. 8, no. 27, pp. 44917–44943, 2017.
- [18] F. Yang, J. Zhang, and H. Yang, “OCT4, SOX2, and NAN OG positive expression correlates with poor differentiation, advanced disease stages, and worse overall survival in HER2,” *OncoTargets and Therapy*, vol. 11, pp. 7873–7881, 2018.
- [19] J. Matsuoka, M. Yashiro, K. Sakurai et al., “Role of the stemness factors SOX2, oct3/4, and nanog in gastric carcinoma,” *Journal of Surgical Research*, vol. 174, no. 1, pp. 130–135, 2012.
- [20] B. W. Kim, H. Cho, C. H. Choi et al., “Clinical significance of OCT4 and SOX2 protein expression in cervical cancer,” *BMC Cancer*, vol. 15, no. 1, p. 1015, 2015.
- [21] Y. Chen, Y. Huang, Y. Huang, J. Chen, S. Wang, and J. Zhou, “The prognostic value of SOX2 expression in non-small cell lung cancer: a meta-analysis,” *PLoS One*, vol. 8, no. 8, Article ID e71140, 2013.
- [22] X. M. Du, L. H. Wang, X. W. Chen, Y. X. Li, Y. C. Li, and Y. W. Cao, “Prognostic value of SOX2 expression in digestive tract cancers: a meta-analysis,” *Journal of Huazhong University of Science and Technology*, vol. 36, no. 3, pp. 305–312, 2016.
- [23] I. V. Lundberg, A. Lofgren Burstrom, S. Edin et al., “SOX2 expression is regulated by BRAF and contributes to poor patient prognosis in colorectal cancer,” *PLoS One*, vol. 9, no. 7, Article ID e101957, 2014.
- [24] T. J. Miller, M. J. McCoy, C. Hemmings, M. K. Bulsara, B. Iacopetta, and C. F. Platell, “The prognostic value of cancer stem-like cell markers SOX2 and CD133 in stage III colon cancer is modified by expression of the immune-related markers FoxP3, PD-L1 and CD3,” *Pathology*, vol. 49, no. 7, pp. 721–730, 2017.
- [25] D. Moher, A. Liberati, J. Tetzlaff, D. G. Altman, and The Prisma Group, “Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement,” *PLoS Medicine*, vol. 6, no. 7, Article ID e1000097, 2009.
- [26] R. Kantor, A. Kim, J. P. Thyssen, and J. I. Silverberg, “Association of atopic dermatitis with smoking: a systematic review and meta-analysis,” *Journal of the American Academy of Dermatology*, vol. 75, no. 6, pp. 1119–1125 e1, 2016.
- [27] D. P. Taggart, R. D’Amico, and D. G. Altman, “Effect of arterial revascularisation on survival: a systematic review of studies comparing bilateral and single internal mammary arteries,” *The Lancet*, vol. 358, no. 9285, pp. 870–875, 2001.
- [28] M. J. Larsen, M. Thomassen, Q. Tan, K. P. Sorensen, and T. A. Kruse, “Microarray-based RNA profiling of breast cancer: batch effect removal improves cross-platform consistency,” *BioMed Research International*, vol. 2014, Article ID 651751, 11 pages, 2014.
- [29] W. E. Johnson, C. Li, and A. Rabinovic, “Adjusting batch effects in microarray expression data using empirical Bayes methods,” *Biostatistics*, vol. 8, no. 1, pp. 118–127, 2007.
- [30] J. IntHout, J. P. Ioannidis, and G. F. Borm, “The Hartung-Knapp-Sidik-Jonkman method for random effects meta-analysis is straightforward and considerably outperforms the standard DerSimonian-Laird method,” *BMC Medical Research Methodology*, vol. 14, no. 1, p. 25, 2014.
- [31] E. Zintzaras and J. P. Ioannidis, “HEGESMA: genome search meta-analysis and heterogeneity testing,” *Bioinformatics*, vol. 21, no. 18, pp. 3672–3673, 2005.
- [32] J. P. T. Higgins, S. G. Thompson, J. J. Deeks, and D. G. Altman, “Measuring inconsistency in meta-analyses,” *British Medical Journal*, vol. 327, no. 7414, pp. 557–560, 2003.
- [33] M. Egger, G. Davey Smith, M. Schneider, and C. Minder, “Bias in meta-analysis detected by a simple, graphical test,” *British Medical Journal*, vol. 315, no. 7109, pp. 629–634, 1997.
- [34] T. Li, P. S. Hu, Z. Zuo et al., “METTL3 facilitates tumor progression via an m6A-IGF2BP2-dependent mechanism in colorectal carcinoma,” *Molecular Cancer*, vol. 18, no. 1, p. 112, 2019.
- [35] J. Zheng, L. Xu, Y. Pan et al., “SOX2 modulates motility and enhances progression of colorectal cancer via the Rho-ROCK signaling pathway,” *Oncotarget*, vol. 8, no. 58, pp. 98635–98645, 2017.
- [36] X. Yan, L. Liu, H. Li, H. Qin, and Z. Sun, “Clinical significance of Fusobacterium nucleatum, epithelial-mesenchymal transition, and cancer stem cell markers in stage III/IV colorectal cancer patients,” *Onco Targets Therapy*, vol. 10, pp. 5031–5046, 2017.
- [37] L. Raghoebir, K. Biermann, M. B. Kempen et al., “Aberrant SOX2 expression in colorectal cancers does not correlate with mucinous differentiation and gastric mucin MUC5AC expression,” *Virchows Archiv*, vol. 465, no. 4, pp. 395–400, 2014.

- [38] K. Ma, X. Pan, P. Fan et al., “Loss of miR-638 in vitro promotes cell invasion and a mesenchymal-like transition by influencing SOX2 expression in colorectal carcinoma cells,” *Molecular Cancer*, vol. 13, no. 1, p. 118, 2014.
- [39] H. J. Lee, D. W. Eom, G. H. Kang et al., “Colorectal micropapillary carcinomas are associated with poor prognosis and enriched in markers of stem cells,” *Modern Pathology*, vol. 26, no. 8, pp. 1123–1131, 2013.
- [40] X. Han, X. Fang, X. Lou et al., “Silencing SOX2 induced mesenchymal-epithelial transition and its expression predicts liver and lymph node metastasis of CRC patients,” *PLoS One*, vol. 7, no. 8, Article ID e41335, 2012.
- [41] J. Neumann, F. Bahr, D. Horst et al., “SOX2 expression correlates with lymph-node metastases and distant spread in right-sided colon cancer,” *BMC Cancer*, vol. 11, no. 1, p. 518, 2011.
- [42] C. W. Ong, L. G. Kim, H. H. Kong et al., “CD133 expression predicts for non-response to chemotherapy in colorectal cancer,” *Modern Pathology*, vol. 23, no. 3, pp. 450–457, 2010.
- [43] K. Liu, B. Lin, M. Zhao et al., “The multiple roles for SOX2 in stem cell maintenance and tumorigenesis,” *Cellular Signaling*, vol. 25, no. 5, pp. 1264–1271, 2013.
- [44] S. Han, T. Huang, X. Wu et al., “Prognostic value of CD133 and SOX2 in advanced cancer,” *Journal of Oncology*, vol. 2019, Article ID 3905817, 12 pages, 2019.
- [45] S. Han, T. Huang, W. Li et al., “Prognostic value of CD44 and its isoforms in advanced cancer: a systematic meta-analysis with trial sequential analysis,” *Frontiers Oncology*, vol. 9, p. 39, 2019.
- [46] G. Y. Liou, “CD133 as a regulator of cancer metastasis through the cancer stem cells,” *The International Journal of Biochemistry & Cell Biology*, vol. 106, pp. 1–7, 2019.
- [47] S. Prasad, S. Ramachandran, N. Gupta, I. Kaushik, and S. K. Srivastava, “Cancer cells stemness: a doorstep to targeted therapy,” *Biochimica et Biophysica Acta (BBA)—Molecular Basis of Disease*, vol. 1866, no. 4, Article ID 165424, 2019.
- [48] C. Saygin, D. Matei, R. Majeti, O. Reizes, and J. D. Lathia, “Targeting cancer stemness in the clinic: from hype to hope,” *Cell Stem Cell*, vol. 24, no. 1, pp. 25–40, 2019.