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REVIEW

Prognostic Significance of Cyclin D1 Expression in Renal Cell Carcinoma: a Systematic Review and Meta-analysis

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Abstract

Previous studies indicated that cyclin D1 shown the potential as a tumor biomarker. However, the prognostic value of cyclin D1 in renal cell carcinoma (RCC) remains controversial. This study investigated the correlation of cyclin D1 expression with the prognostic and clinicopathological features in RCC patients. We systematically searched the database of PubMed, Embase, Cochrane, and Web of Science updated on November 26, 2017. Eighteen studies with 2282 patients satisfied the inclusion criteria. Results demonstrated that cyclin D1 overexpression in RCC showed significant favorable prognostic impact on disease-free survival (DFS) (HR 0.57, 95% CI: 0.43–0.74) and disease-specific survival (DSS) (HR 0.59, 95% CI 0.41–0.85) without significant heterogeneity. In subgroup of clear cell RCC, the prognostic effect on DFS was robust and the pooled HR was 0.39 (95% CI: 0.27–0.57). However, no association between overall survival (OS) and cyclin D1 expression was observed. Stratified analysis in DFS studies by sample size, staining patterns race and metastasis status showed similar results. Otherwise, cyclin D1 overexpression predicted a reduced prevalence of high TNM stage (T3 + T4) (OR 0.63, 95% CI: 0.40–0.99), high-grade tumor (G3 + G4) (OR 0.51, 95% CI: 0.31–0.81) and large tumor size (OR 0.35, 95% CI: 0.19–0.62). Our meta-analysis indicated that cyclin D1 overexpression could predict the favorable prognosis in patients with RCC.

Keywords Cyclin D1 · Renal cell carcinoma · Prognosis · Meta-analysis

Introduction

Renal cell carcinoma (RCC), as the second leading cause of mortality in the urological malignant tumor, is responsible for 2% of all human malignancies [1]. Despite radical surgical resection, 25% patients develop disease recurrence or metastasis eventually [2]. Target and biology therapy was advanced in recent years, but far from expectation due to the heterogeneity of RCC [3]. Prognostic biomarkers could guide individualized treatments, assess therapeutic responses and optimize follow-up. Despite the potential that prognostic biomarker holds, scarcely any marker has reached the clinical practice. The deficiency of comprehensive prospective studies impairs the utility of biomarkers. Therefore, effective assessment of the prognostic value of biomarkers is urgently needed.

During the last two decades, great scientific efforts have focused on the cell cycle regulation in tumorigenesis [4]. As a critical regulator of G1/S transition, cyclin D1 is generally considered as an oncogene [5] and thus intrigues much interest. Cyclin D1 Overexpression was observed in extensive tumor types [4, 6], including RCC [7, 8]. Many quantitative studies demonstrated that cyclin D1 overexpression was associated with poor prognosis in various malignancies, such as breast cancer [9] and colorectal cancer [10]. However, conflicting results from meta-analysis were reported constantly [11, 12]. Interestingly, Tobin et al. proposed that cyclin D1 downregulation increased the invasion of breast cancer mediated by epithelial-mesenchymal transition (EMT) [13] and another study demonstrated that cyclin D1, which could bypass its conservative oncogene function, directly inhibit

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oncogenic signal transducer and activator of transcription 3 (STAT3) to improve outcome [14].

Given the complicated multiple roles of cyclin D1, we speculated that cyclin D1 may exert cancer-specific functions. In renal cell carcinoma, the prognostic impact of cyclin D1 has been investigated in many studies. Nevertheless, the results remain controversial. Lacking corroboration from comprehensive prospective cohort study, small sample observational studies are far from being persuasive. Thus, we performed this systematic review to investigate the correlation of cyclin D1 expression with the prognosis in patients with RCC.

Material and Methods

Search Strategy

We performed a systematic literature search in the databases of PubMed, Embase, Cochrane library and Web of Science updated on November 26, 2017. The guideline of Preferred Reporting Item for Systematic Reviews and Meta-analyses (PRISMA) statement was followed [15] (Online Resource Table S1). The search strategy was the combination of cyclin D1 and renal cell carcinoma (subject terms plus free words). No restriction of title/abstract in search strategy was employed to avoid literature omission. References of retrieved articles were manually reviewed to identify the relevant articles.

Inclusion and Exclusion Criteria

Studies meeting the following inclusion criteria were included in our systematic review: (i) detected the expression of cyclin D1 in the primary clinical sample of RCC tissue by immunohistochemistry (IHC) or tissue microarrays (TMA); (ii) reported the DFS, OS, and DSS in RCC regarding cyclin D1 expression; (iii) investigated the association between clinicopathological features of RCC and cyclin D1 expression; (iv) When the same subject population was used in several studies, the more updated study or study with larger sample was included. The exclusion criteria were listed as follows: (i) reviews or case reports; (ii) studies reported animal or cell line models; (iii) studies did not investigate the correlation of cyclin D1 expression with prognosis or clinicopathological features of RCC; (iv) the subject number was less than 30.

Data Extraction

Two authors (Li ZY and Liu JK) independently reviewed all the articles based on search strategy. Disagreements were resolved by consensus. As for some conference abstracts and articles with insufficient data, the corresponding author and first author were contacted. The cut-offs of cyclin D1 were defined based on the standard in the original study. Detailed items were listed in Table 1 and Online Resource Table S2.

Quality Assessment

Two authors (Li ZY and Liu JK) independently assessed the literature quality of all studies included in accordance with the Newcastle-Ottawa Scale (NOS) [32]. Unsettled discrepancies were resolved by discussions among authors.

Statistical Analysis

The statistical analysis was conducted according to guidelines proposed by the Meta-Analysis of Observational Studies in Epidemiology group [33]. The prognostic outcomes of our interest were DFS, OS, and DSS. Hazard ratio (HR) and the corresponding 95% confidence interval (CI) were deployed to estimate the prognostic efficiency of cyclin D1 on RCC. HR was estimated as high cyclin D1 expression compared to low expression. HR>1 represents that cyclin D1 overexpression is associated with better prognosis as compared to that of the low expression, while HR<1 represents the opposite result. When the necessary data for HR was not provided, Kaplan-Meier curves were read by Engauge Digitizer version 4.1 to extrapolate HR according to the method developed by Tierney et al. [34]. Regarding the pooled analysis of clinicopathological characteristics and cyclin D1 expression, odds ratio (OR) and its 95%CI were pooled. Between-study heterogeneity was evaluated using Q-test and I^2-statistics. Random-effects model was adopted when heterogeneity was significant. The source of between-study heterogeneity was explored by subgroup analysis. Sensitivity analyses were performed to assess influence of single study on the overall meta-analysis by omitting one study at a time. Besides, publication bias was measured using Begg's rank correlation test and Egger's regression test. Duval and Tweedie trim-and-fill method was employed to examine the robustness of pooled results. P values involved are two-tailed and $P < 0.05$ was considered significant. All statistical analyses were implemented by STATA 12.0 (Stata Corporation, College Station, USA).

Results

Search Results and Characteristics of Eligible Studies

Stepwise search strategy was elaborately exhibited as Fig. 1. Eventually, 18 observational studies were enrolled in the qualitative synthesis [7, 8, 16–31] and 16 studies [8, 16–30] with 2283 patients were included in meta-analysis. Seven studies reported DFS [16, 17, 19, 22–25], four studies reported OS [18, 23, 25, 26], and DSS was obtained from three studies [8,

Table 1 Characteristics of included studies

Author	Year	race	Design	Period	Median*/Mean# follow-up (month)	N	Median*/Mean# age (year)	Histology	Cutoff value (high/low)	Blind to outcomes	Staining patterns	Outcome	NOS
Hedberg [8]	2003	Caucasian	Prospective cohort	1982–1997	* 97 (38–209)	174	* 65.1 (25–87)	ccRCC	>5 positive cells per core (92/82)	NA	TMA/ IHC (nucleus)	DSS	7
Furukawa [16]	2016	Asian	Retrospective cohort	2009–2011	# 15.1	39	61 (36–77)	ccRCC	>10%(18/21)	YES	IHC(no validated specificity)	DFS	7
Haddad [17]	2017	Caucasian	Retrospective cohort	1997–2010	*63.5(24–85.3)	367	TC: *56(27–85) VC: *59(17–85)	ccRCC	score ≥ 8.5	YES	TMA/ IHC (nucleus)	DFS	7
Portela [18]	2011	Caucasian	Case control	2000–2004	* 31	30	(48–71)	ccRCC	>6% (14/16)	NA	IHC (nucleus)	OS	5
Lima [19]	2014	Caucasian	Prospective cohort	2005–2010	* 20 (1–79)	109	# 58.5 (12–89)	ccRCC 78 non-ccRCC 31	>30%(57/52)	NA	IHC (nucleus)	DFS	8
Biswas[20]	2012	Caucasian	Retrospective cohort	1983–1999	* 124 # 126	112	* 64 (27–87)	ccRCC	median histoscore	YES	TMA/ IHC (nucleus)	DSS	7
Phuoc [21]	2007	Asian	Retrospective cohort	1987–2003	* 69.3 (3.6–215.2)	119	* 61 (23–86)	ccRCC	≥10%(76/43)	YES	IHC (nucleus)	DSS	8
Allory [22]	2005	Caucasian	Retrospective cohort	1989–2002	# 26 (2–57)	72	# 63 (24–92)	ccRCC	≥10%(44/28)	NA	TMA/ IHC (nucleus)	DFS	8
Stubbs [23]	2017	Caucasian	Retrospective cohort	2006–2012	>5 years	59	* 61 (34–86)	ccRCC 50 non-ccRCC 9	>10%	YES	IHC(no validated specificity)	DFS,OS	6
Kusuda [24]	2013	Asian	Retrospective cohort	2008–2009	* 14.2 (1.5–21.6)	45	* 66 (42–83)	ccRCC	>20%(31/14)	YES	IHC (nucleus)	DFS	7
Bodnar [25]	2013	Caucasian	Prospective cohort	NA	<5 years	53	* 60 (41–78)	ccRCC 54 non-ccRCC 4	score > 5(23/30)	YES	IHC(no validated specificity)	DFS, OS	7
Su [26]	2013	Asian	Prospective cohort	1998–2011	* 34 (3.0–90.9)	90	NA	mixed	>20%(33/57)	YES	IHC(no validated specificity)	OS	7
Dahinden [27]	2010	Caucasian	Prospective cohort	NA	# 51.9 (0.1–229)	804	# 62.7 (15–88)	ccRCC	≥5%	NA	TMA/ IHC (nucleus)	NA	7
Wang [28]	2012	Asian	Case control	1998–2010	None	67	* 55 (32–67)	ccRCC	>25%	NA	IHC (nucleus)	None	7
Migita [29]	2002	Asian	Prospective cohort	1989–2000	# 35 (2–94)	67	# 60.7	ccRCC	≥10%(29/38)	YES	IHC (nucleus)	DSS	9
Ge [30]	2007	Asian	Prospective cohort	1995–2002	# 51 (15–60)	76	# 55 (17–82)	ccRCC	≥10%(42/34)	NA	IHC (nucleus)	NA	6
Aaltomaa [7]	1999	Caucasian	Prospective cohort	1968–1985	# 116	82	# 61 (22–82)	NA	strong positivity	YES	IHC (nucleus)	DFS	8
Hsu [31]	2012	Asian	Retrospective cohort	1988–2006	228	284	59.4 ± 13.8	ccRCC 230 non-ccRCC 54	score > 68.5% (160/124)	YES	TMA/ IHC (nucleus)	DFS,OS	8

Mixed referred to mixed histology types including clear cell, papillary, chromophobe, unclassified RCC

Abbreviations: *NA* not available, *DFS* disease-free survival, *OS* overall survival, *DSS* disease-specific survival, *VC* validation cohort, *TC* training cohort, *IHC* immunohistochemistry, *TMA* tissue microarrays, *NOS* Newcastle-Ottawa Scale, *ccRCC* clear cell renal cell carcinoma

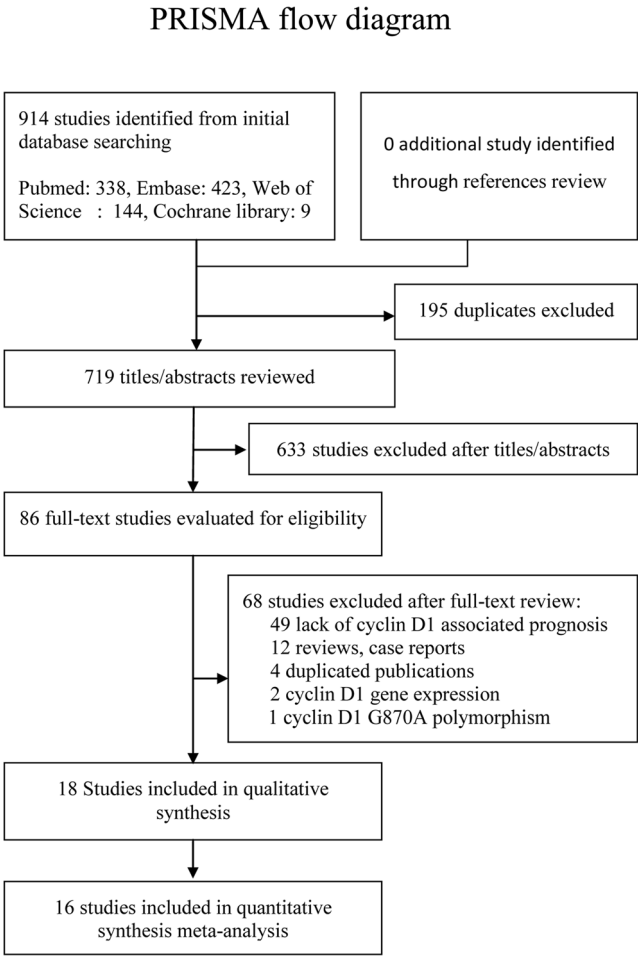

Fig. 1: Flow diagram of study selection

20, 21]. All characteristics of 18 eligible studies were listed in Table 1 and Online Resource Table S2.

Methodological Quality of the Studies

Newcastle-Ottawa Scale (NOS) was followed to assess the methodological quality of included studies. As shown in Table 1 and Online Resource Table S3, the NOS scores ranged from 5 to 9 with a mean of 7.17. As for the item of representativeness of cohort or cases in the NOS scale, 11 studies failed to score. With regard to the item of adequacy of follow up, all the cohort studies failed to score except two cohorts [21, 29]. Other items reached agreement unanimously during the assessment.

Impact of Cyclin D1 Expression on DFS in RCC

Results showed that cyclin D1 overexpression was associated with favorable DFS in RCC (HR 0.57, 95% CI: 0.43–0.74, $P < 0.001$, fixed-effects) without substantial heterogeneity ($P_h = 0.159$, $I^2 = 33.7\%$) (Fig. 2a). Subgroup analyses indicated patients with clear cell RCC (ccRCC) demonstrated better DFS in high cyclin D1 expression group than that in low expression group. Prognostic effect was robust (HR 0.39, 95% CI: 0.27–0.57, $P < 0.001$) with no heterogeneity ($P_h = 0.53$, $I^2 = 0.0\%$) (Fig. 2b). Besides, cyclin D1 overexpression showed a significant favorable prognosis in both IHC group (HR 0.68, 95% CI: 0.50–0.94, $P = 0.018$; $P_h > 0.4$, $I^2 = 0.0\%$) and TMA group (HR 0.32, 95% CI: 0.19–0.56, $P < 0.001$; $P_h > 0.4$, $I^2 = 0.0\%$). Cyclin D1 overexpression highly correlated with better DFS in subgroup of non-metastatic (HR 0.36, 95% CI: 0.20–0.65, $P = 0.001$) and mixed-metastatic (HR 0.39, 95% CI: 0.22–0.70, $P = 0.001$). Considering the heterogeneity of cut-offs among studies, we performed the subgroup analyses according to the cut-offs. Results showed that higher cyclin D1 tended to have better DFS in all subgroups (Fig. S1A and B). Moreover, subgroup analyses dichotomized by race, follow-up length, blind to outcomes and NOS score were also performed. Except for the subgroup of Asian, cyclin D1 overexpression in other subgroups was associated with favorable DFS. However, the heterogeneity of pooled results from these subgroups was larger compared to DFS in RCC. Corresponding data in detail was listed in Online Resource Table S4.

Impact of Cyclin D1 Expression on OS in RCC

Overall four studies including 232 cases were assessed for the impact of cyclin D1 expression on the OS in RCC. As illustrated in Fig. 2c, no significant correlation between cyclin D1 expression and OS was observed. Between-studies heterogeneity was remarkable ($P_h = 0.009$, $I^2 = 74.1\%$). In the subgroup of cut-off >6% or>10%, cyclin D1 overexpression had worse OS. Whereas in the subgroup of cut-off >20%, cyclin D1 overexpression had better OS (Fig. S1C). Besides, cyclin D1 expression showed no prognostic impact on OS in any other subgroup (Online Resource Table S4).

Outcome Effect of Cyclin D1 Expression on DSS in RCC

Prognostic effect of cyclin D1 expression on DSS in RCC was also evaluated in three cohorts with 405 cases. The pooled HR was 0.59 (95% CI 0.41–0.85, $P = 0.004$, fixed-effects), without substantial heterogeneity ($P_h = 0.234$, $I^2 = 31.2\%$) (Fig. 2d). Besides, in the subgroup of cut-off >10% and cut-off >5 positive cells/core, cyclin D1 overexpression showed a significant favorable prognosis (Fig. S1D).

Prognostic Impact of Cyclin D1 Expression in Qualitative Studies

In accordance with the inclusion and exclusion criteria, we included four studies which reported inadequate survival data for meta-analysis [7, 27, 29, 31]. Migita et al. found that low cyclin D1 expression tended to decrease survival of 67

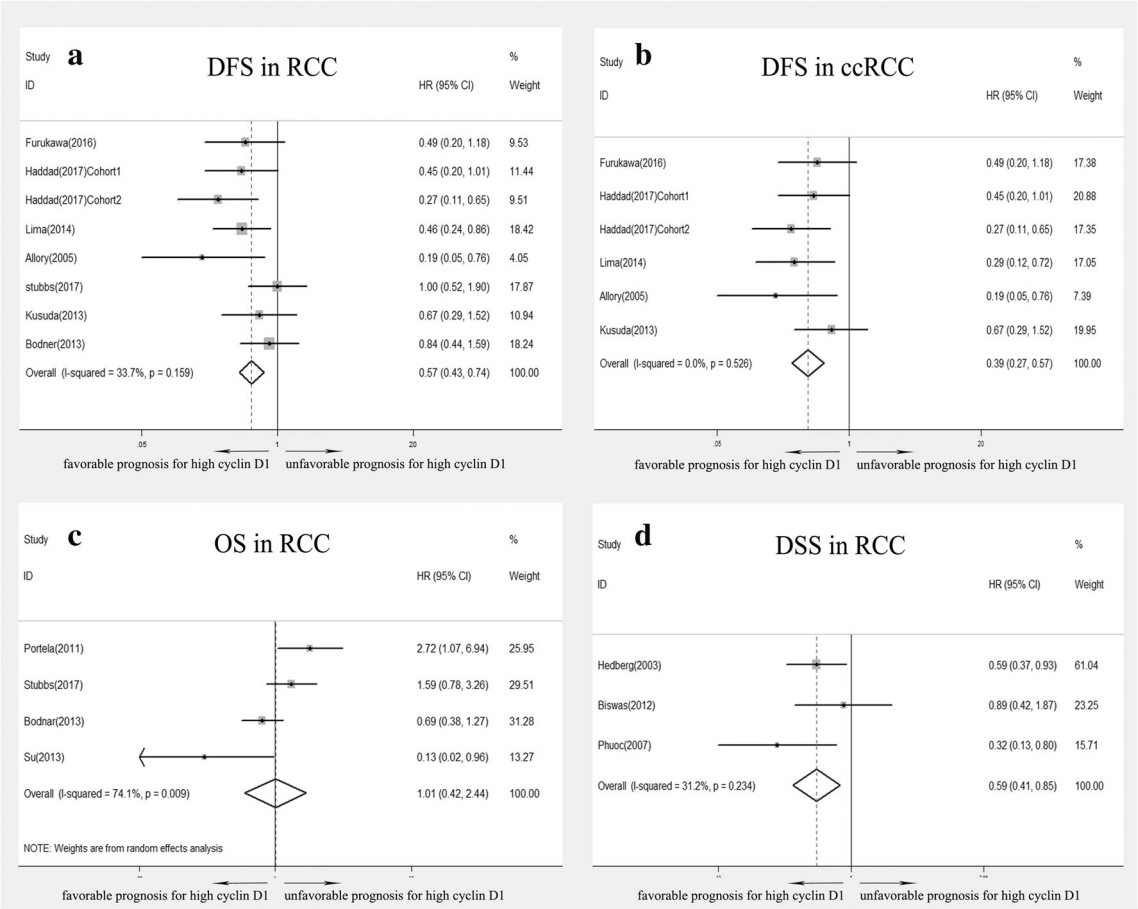

Fig. 2: Forest plots of the DFS (**a**), OS (**c**), DSS (**d**) in RCC and DFS (**b**) in ccRCC

patients with ccRCC [29]. Two studies arrived at statistical insignificant results [7, 27]. Interestingly, the study of Hsu et al. declared that high cyclin D1 served as a risk factor for the RCC prognosis [31]. However, the adoption of risk ratio and extreme long follow-up (19 years) led to a high risk of bias.

Correlation of Cyclin D1 with Clinicopathological Characteristics

The associations between cyclin D1 expression and clinicopathological characteristics were illustrated in Table 2. Lower TNM stage was observed in patients with cyclin D1 overexpression (OR 0.63, 95% CI: 0.40–0.99, $P = 0.045$, fixed-effects). Overexpression of cyclin D1 also predicted a reduced prevalence of histologic high-grade tumor (OR 0.51, 95% CI: 0.32–0.81, $P = 0.005$; $P_h = 0.067$, $I^2 = 51.5\%$). Based on different cut-offs, we conducted subgroup analyses. Cyclin D1 overexpression tended to have a lower histologic stage in any cut-off subgroups. However, in the subgroup of cut-off >10%, no significant correlation between cyclin D1 expression and TNM stage was observed (Table 2). Besides, the difference of tumor size regarding the cyclin D1 expression was also investigated. Result showed that cyclin D1 overexpression had a remarkable correlation with smaller primary tumor size in higher quality (NOS ≥ 7) studies (OR 0.35, 95% CI: 0.19–0.62, $P < 0.001$; $P_h = 0.729$, $I^2 = 0.0\%$).

Sensitivity Analyses

Sensitivity analyses were performed to assess the robustness of pooled results (Online Resource Fig. S2–5). Omitting any individual cohort did not show any appreciable change in DFS. There was no significant difference in pooled results when we adopted random-effects model rather than fixed-effects model. Moreover, sensitivity analyses about OS, DSS, and DFS in ccRCC demonstrated that variation wasn't introduced in pooled HRs after deleting any cohort.

Publication Bias

No publication bias was observed in DFS studies and DFS of ccRCC studies according to Begg's test ($P = 0.266$; $P = 0.133$, respectively, Fig. 3). However, publication bias in egger's test was inconsistent ($P = 0.044$; $P = 0.155$, respectively). Thus, trim-and-fill method was conducted. The results showed that

Table 2 Meta-analysis of correlation of cyclin D1 expression with clinicopathological characteristics of RCC

Categories or subgroups	No. of studies	No. of patients	Model	Outcome		Heterogeneity		
				OR (95% CI)	*P*	Q	P_h	I^2 (%)
TNM stage (III+ IV vs. I + II)	5	409	fixed	0.629 (0.400–0.990)	0.045	2.55	0.636	0.0
Cutoff value								
>20% or > 25% or > 30%	3	266	fixed	0.541 (0.309–0.946)	0.031	1.72	0.423	0.0
≥10%	2	143	fixed	0.850 (0.389–1.859)	0.685	0.03	0.855	0.0
Histologic stage (3 + 4 vs. 1 + 2)	6	1294	random	0.506 (0.315–0.813)	0.005	10.31	0.067	51.5
1. Cutoff value								
>25% or > 30%	2	176	fixed	0.332 (0.174–0.634)	0.001	0.47	0.493	0.0
≥5% or ≥ 10%	3	946	fixed	0.783 (0.578–1.060)	0.114	0.03	0.986	0.0
>5 positive cells/core	1	172	fixed	0.250 (0.101–0.615)	0.003	–	–	–
2.Standard								
Fuhrman	3	252	fixed	0.438 (0.260–0.739)	0.002	2.62	0.270	23.5
non-Fuhrman	3	1042	random	0.541 (0.249–1.177)	0.121	5.56	0.062	64.0
3.Sample size								
≥109	3	1084	random	0.411 (0.175–0.964)	0.041	9.70	0.008	79.4
<109	3	210	fixed	0.630 (0.340–1.166)	0.141	0.60	0.740	0.0
4.race								
Caucasian	3	1084	random	0.411 (0.175–0.964)	0.041	9.70	0.008	79.4
Asian	3	210	fixed	0.630 (0.340–1.166)	0.141	0.60	0.740	0.0
Tumor size (large vs. small)	4	319	random	0.569 (0.212–1.528)	0.263	10.86	0.013	72.4
NOS								
≥7	3	243	fixed	0.348 (0.194–0.623)	0.000	0.63	0.729	0.0
<7	1	76	fixed	2.593 (0.877–7.667)	0.085	–	–	–
Age (old vs. young)	3	252	fixed	1.221 (0.713–2.091)	0.467	0.24	0.889	0.0

Abbreviations: *OR* odds ratio, *CI* confidence interval, *N* number, *NOS* Newcastle-Ottawa Scale, *RCC* renal cell carcinoma

no trim was necessary in every group of DFS, DFS of ccRCC and DSS, suggesting that no obvious publication bias was detected.

Discussion

In the present systematic review, we initially evaluated the correlation of cyclin D1 expression with the prognosis of RCC and clarified the clinical utility of this biomarker. A total of 16 studies with 2283 patients were included to exhibit the comprehensive quantitative review of available evidence. Results demonstrated that cyclin D1 overexpression showed a significant favorable prognostic impact on patients with RCC. No publication bias was observed and sensitivity analyses confirmed its robustness. Besides, we found cyclin D1 overexpression could predict reduced prevalence of histologic high-grade tumor, high TNM stage, and large size, indicating

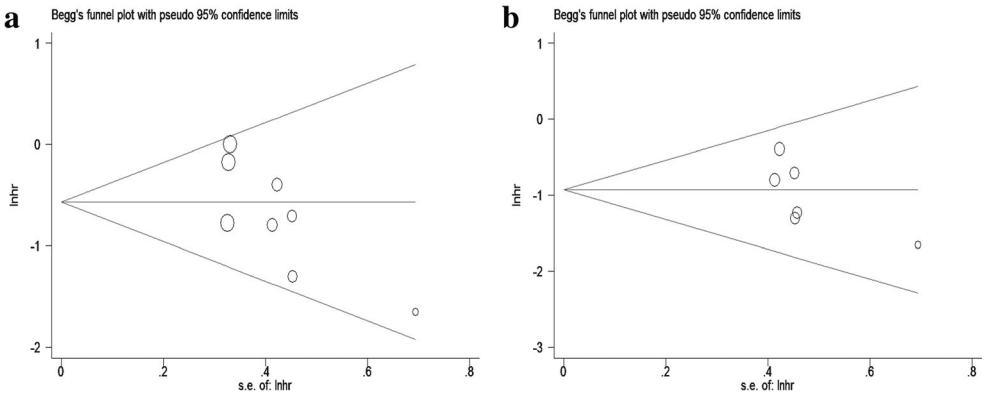

Fig. 3: funnel plots of DFS in RCC (**a**) and DFS in ccRCC (**b**)

that malignant potential was impaired by cyclin D1 upregulation.

As a critical cell cycle protein, the classic role of cyclin D1 is promoting G1/S-phase transition in complex with cyclin-dependent kinase (CDK) 4/6 [35]. Our finding showed that cyclin Dl has a favorable prognostic impact on RCC, which was consistent with the results of some studies reporting that cyclin Dl has functions besides oncogenic. Jirawatnotai et al. have demonstrated that cyclin D1 could mediate homologous recombination-mediated DNA repair. Human cancer cells failed to recruitment of RAD51 to damaged DNA when cyclin D1 was downregulated, and thus increased its sensitivity to radiation [36]. Previous study also found that cyclin D1 upregulation prolonged S-phase in breast cells via retinoblastoma tumor suppressor protein (pRB) and proliferating cell nuclear antigen (PCNA) mediated DNA synthesis or repair [37]. Gillett et al. proposed that the loss of cyclin D1 represented the mutations of retinoblastoma gene (RB). Lacking RB leads to the overexpression of p16, and thus the growth of tumor is inhibited [38]. Besides, cyclin Dl could suppress proliferation of diploid fibroblasts [39] and play a part in programmed cell death [40]. These findings indicated that cyclin D1 had the molecular background to impair the malignant potential of RCC.

Given the inherent heterogeneity of RCC, it is expected that distinctive survival-related expression of cyclin D1 could be found among RCC subtypes. Our results showed that overexpression of cyclin D1 strongly correlated with ameliorated prognosis in the studies recruited ccRCC. The impact was more robust (HR 0.39, 95% CI: 0.27–0.57) with less heterogeneity ($P_h = 0.53$, $I^2 = 0.0\%$) compared to RCC studies. In the systematic review, Hedberg et al. [8] and Alloy et al. [22] both observed that cyclin D1 overexpression showed favorable prognostic impact in ccRCC, whereas no significant impact in other subtypes including papillary and chromophobe RCC. Likewise, the study of Lima et al. reported obviously better survival in ccRCC group than that in RCC group [19]. Besides, we found cyclin D1 expression in ccRCC was significantly higher than that in papillary and chromophobe RCC [8, 19]. Von Hippel-Lindau (VHL) mutation, as a distinctive feature of ccRCC, may account for the cyclin D1 impact. After degradation by VHL, hypoxia inducible factor α (HIF-α) could regulate cyclin D1 at transcription level [27]. Thus, elucidating the mechanism of distinctive role of cyclin D1 in differentiated RCC subtypes would facilitate prognosis assessment and promote understanding of tumorigenesis, which however beyond the range of our study.

In the subgroup analysis of DFS, we found that studies with large sample size were inclined to report significant favorable prognosis at cyclin D1 overexpression. Based on the normal distribution pattern, larger sample predicts more accurate and authentic results, which is supportive for our conclusion. Besides, results showed localized RCC patients benefited more from cyclin D1 overexpression than the patients with metastatic RCC. It has been demonstrated that cyclin D1 overexpression was closely related to decreased invasion and EMT in vitro [13]. Therefore, we speculated that cyclin D1 overexpression in primary tumor may inhibit the invasion and metastasis of RCC. Additionally, the staining patterns seem to partly interpret the heterogeneity between studies. TMA, as an advanced method for high-flux screening of biomarker, has been employed extensively [41]. The representative of microcores for the whole tissue section remains its prioritized concern. Similarly, our result showed significant heterogeneity between subgroups of IHC and TMA. Thus, discrepancies from different staining patterns deserve much attention in future studies.

Surprisingly, no prognostic impact of cyclin D1 was observed in OS studies. Further subgroup analyses could not interpret its heterogeneity. Small sample (mean 58) and low NOS score (mean 6.25) make the result prone to bias. Furthermore, OS provides less accurate and persuasive conclusion than DSS due to confounding factors of other diseases. Thus, this unexpected result needs to be further investigated.

Methodology reproducibility of included studies dominates the stability of meta-analysis. Despite the fact that all the studies involving prognostic synthesis were cohorts, quite a large proportion of studies were of retrospective nature for the follow-up data. Prospective-retrospective design, proposed by Simon et al, emphasized its RCT attribute of prospective follow-up data collection [42]. Thus, purely retrospective design may introduce bias to the conclusion. Besides, the variation of definition of biomarker expression could also influence reproducibility. As a result, the cut-offs implicating in our included studies varied to a significant extent ranging from 5 to 30% [17]. This discrepancy could be interpreted not only from technical perspective including the usage of various antibodies and different staining patterns, but also from subjectivity of the observer. Among all included studies, most of them defined cut-offs arbitrarily and only five determined cut-offs based on distribution of data, under which circumstance the selection bias may be inevitable. Considering that the heterogeneity of cut-offs may affect the availability of cyclin D1 as a predictive biomarker, more standardized methodology is therefore expected in future studies. Furthermore, the adoption of blind method may be another concern. Seven studies did not mention the use of blind, which were at high risk of report bias. Given all the above considerations, a standardized methodology regarding biomarker study remains to be established in further researches.

In conclusion, our meta-analysis initially demonstrated that cyclin D1 overexpression correlated with favorable prognostic impact and impaired malignant potential in RCC. Besides, we highlighted its robust and stabilized prognostic impact in ccRCC. However, strengths should be balanced against the limitations mentioned in discussion. Therefore, studies of high

quality are needed to further evaluate the promising role of cyclin D1 as a prognostic biomarker.

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Compliance with Ethical Standards

This research does not involve Human Participants and/or Animals.

This article does not contain any studies with human participants performed by any of the authors. No informed consent is needed.

Conflict of Interest The authors declare that they have no conflict of interest.

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