

Prognostic Value of CD63 Expression in Solid Tumors: A Meta-analysis of the Literature

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Abstract. *Background:* CD63 has been described as a key factor in extracellular vesicle production and endosomal cargo sorting, and there have been certain reports suggesting an association between CD63 expression and survival in patients with tumors including gastric, colon and lung cancer. However, the prognostic value of CD63 expression remains contradictory. Hence, we performed this meta-analysis to assess the prognostic value of CD63 expression in solid tumors. *Materials and Methods:* Eligible studies were collected by searching the PubMed, Embase and Cochrane libraries. The hazard ratio (HR) with 95% confidence interval (CI) were evaluated to reveal the association between CD63 expression and survival in solid tumors. *Results:* Five studies with a total of 1,454 patients were included. The HR evaluating CD63 expression on survival was 1.34 (95%CI=0.92-1.97, $p=0.129$). In subgroup analysis, the HRs of lung cancer and other tumors were 0.50 (95% CI=0.32-0.77, $p=0.002$) and 2.16 (95% CI=1.93-2.42, $p<0.001$) respectively. CD63 expression was significantly associated with poor disease-specific survival (HR=1.69, 95% CI=1.15-2.49, $p=0.008$), but not with disease-free survival and overall survival. Also, there was a significant association between CD63 expression with poor survival in the group of sample size more than 150 patients (HR=2.15,

$95\% CI=2.92-2.41$, $p<0.001$), but not in the group of sample size with fewer than 150 patients. *Conclusion:* This meta-analysis suggested that CD63 expression may be a potential prognostic marker in solid tumors.

Exosomes are small membrane-bound vesicles that are released from various cells into the extracellular spaces (1, 2). Such vesicles contain proteins, lipids and nucleic acids, which can be taken up by neighboring or distant cells and subsequently modulate recipient cells (1, 2). Recently, it has been reported that exosomes from tumor cells might be involved in tumor progression by modulating the tumor microenvironment, *e.g.* metastatic niche formation and angiogenesis (3).

The tetraspanin protein CD63 has been described as a key factor in extracellular vesicle production and endosomal cargo sorting, and there have been reports evaluating the association between CD63 expression and tumor existence (4). Moreover, some studies have suggested that CD63 expression is associated with survival in patients with tumors (3, 5-8). However, the prognostic value of CD63 expression remains contradictory or inconclusive. Hence, we conducted this meta-analysis to investigate the prognostic value of CD63 expression in solid tumors.

Materials and Methods

Literature search. We performed a literature search in PubMed, Embase and Cochrane library up to March 15, 2020 using the following terms: (CD63) and (cancer or tumor or carcinoma or neoplasm or malignancy) and (prognostic or predict or prognosis or survival or outcome). All articles were screened for exclusion and review articles were screened to find eligible studies.

Inclusion and exclusion criteria. Inclusion criteria for this study were as follows: (i) CD63 expression analysis was conducted in

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Key Words: CD63, meta-analysis, prognosis, solid tumors.

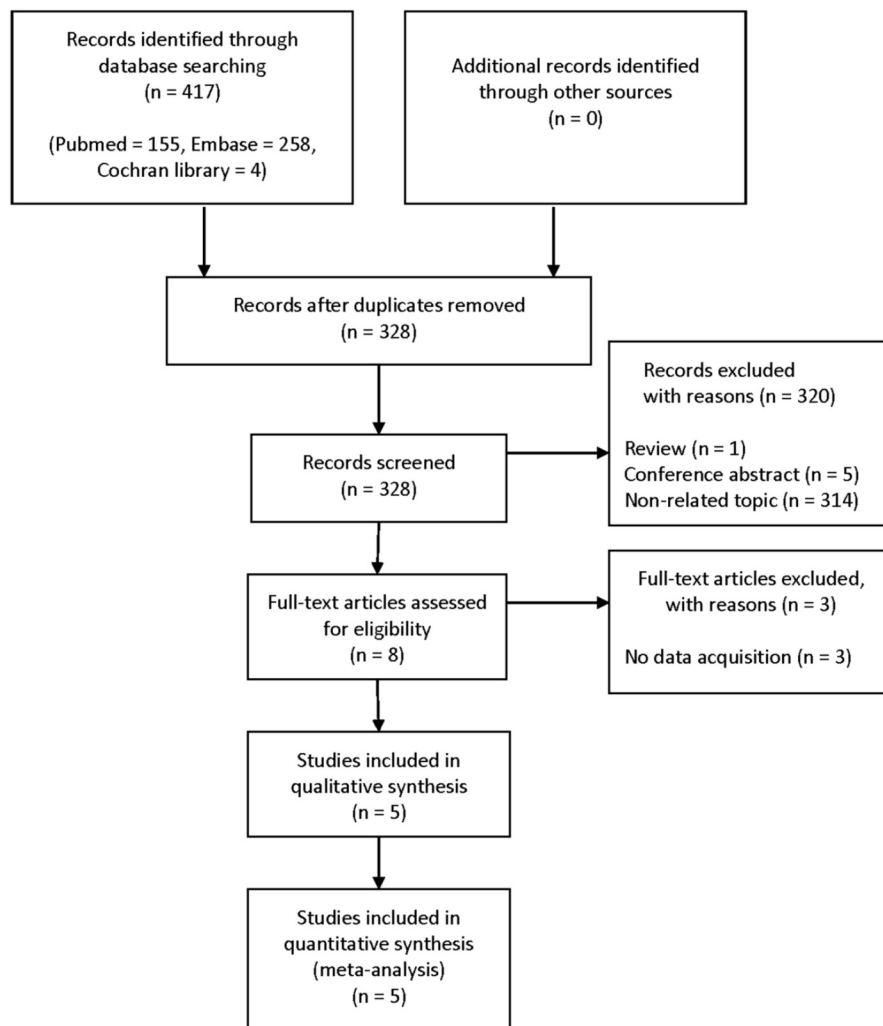


Figure 1. Flow diagram for selection of studies to be included in the meta-analysis.

human tumor tissue, (ii) the association between CD63 expression and survival was evaluated, (iii) the hazard ratio (HR) with 95% confidence interval (CI) was provided. Exclusion criteria were as follows: (a) duplicate studies, (b) conference abstracts, case reports, reviews, letters, and non-English articles.

Data extraction and quality assessment. Two independent authors collected data from the full texts of included articles. The collected data were as follows: first author, publication year, country, cancer type, sample size, sex and median age of patients, study period, follow-up period, survival outcomes, CD63 expression associated with poor prognosis, cut-off value of CD63 expression, and HR with 95% CI for survival. Any differences in the data extraction were resolved by consensus.

The quality of included articles was evaluated using the Newcastle-Ottawa Scale (NOS). Article with score greater than 6 was considered as a high quality. Two independent authors performed quality assessment of included articles.

Statistical analysis. All data were analyzed using StataSE12 (Stata, College Station, TX, USA). $p < 0.05$ was regarded as statistically significant. Cochran's Q and I^2 statistics were used to evaluate the heterogeneity among the included articles. HR with 95% CI was calculated for assessing the prognostic value of CD63 expression in solid tumors. Funnel plot with Egger's test was also performed to reveal the publication bias. And the sensitivity analysis was conducted to explore possible explanations for heterogeneity.

Results

Study selection and characteristics. A total of 417 studies were reviewed electronically, 320 of which were excluded for the reasons explained in Materials and Methods. Eight full-text articles were assessed for eligibility, and finally five articles were available for this meta-analysis (Figure 1).

Table I. Basic characteristics of the included studies.

Study	Country	Cancer type	Sample size	Gender (M/F)	Mean age (years)	Study period	Follow-up (months)	Survival outcome	CD63 detection	CD63 expression associated with poor prognosis	Cut-off value of CD63 expression	Survival analysis	NOS
Kaprio <i>et al.</i> (2020)	Finland	Colorectal cancer and metastasized colorectal cancer	582	NA	66.2	1990-2001	Median 85.2	DSS	IHC	Positive expression	Positive expression (low, moderate and high)	MVA	8
Koh <i>et al.</i> (2019)	South Korea	Non-small cell lung cancer	133	111/22	NA	2002-2009	NA	DSS, DFS	IHC	Low expression	Staining scores with intensity and proportion (<4)	UVA (DSS) MVA (DFS)	7
Miki <i>et al.</i> (2018)	Japan	Gastric cancer	595	331/264	NA	NA	NA	OS	IHC	Positive expression	Staining scores with intensity and proportion (≥2)	MVA	7
Lewitowicz <i>et al.</i> (2016)	Poland	Gastro-intestinal stromal tumor	54	28/26	62.6	1998-2015	Median 36	DFS	IHC	High expression	Moderate and strong expression	MVA	8
Kwon <i>et al.</i> (2007)	South Korea	Lung adenocarcinoma	90	67/23	62.6	1994-2001	NA	OS	IHC	Negative expression	Negative expression	MVA	7

DFS, Disease-free survival; DSS, disease-specific survival; IHC, immunohistochemistry; MVA, multivariate analysis; NA, not available; NOS, Newcastle-Ottawa Scale; OS, overall survival, UVA, univariate analysis.

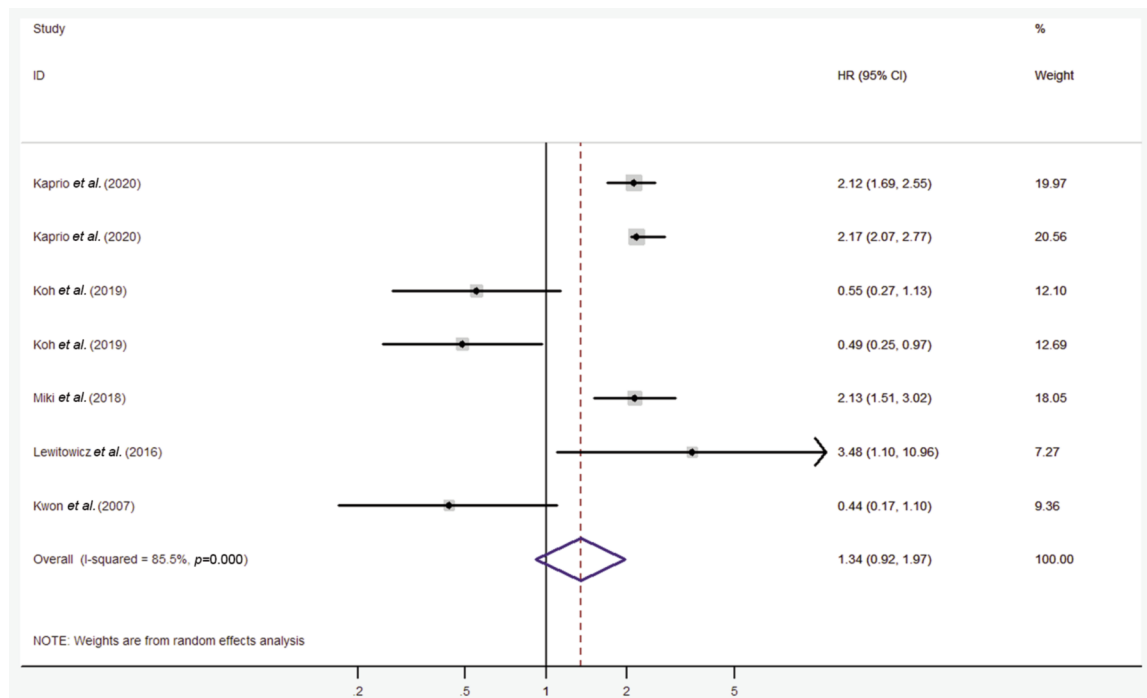


Figure 2. Forest plot of the association between CD63 expression and survival.

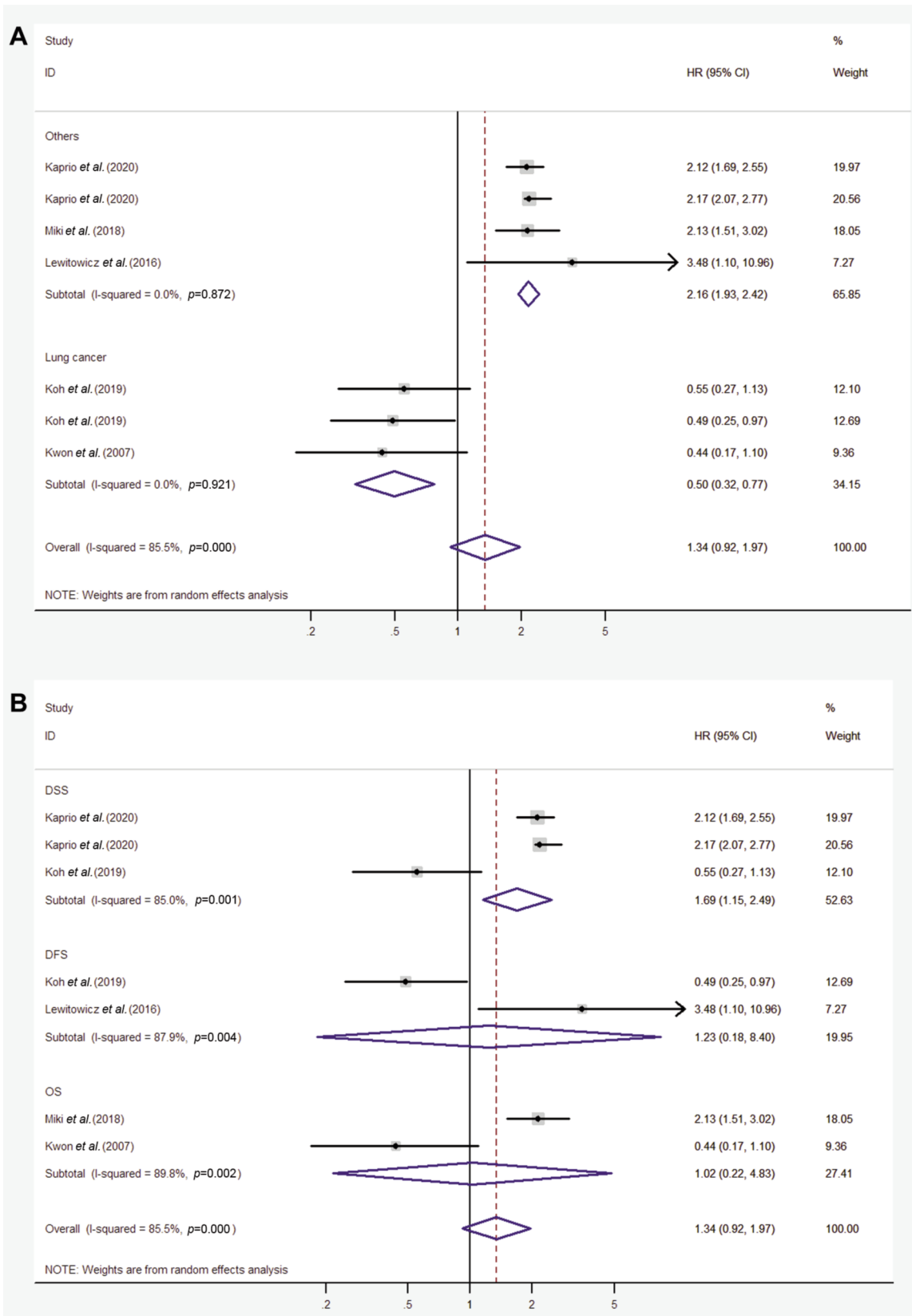


Figure 3. Continued

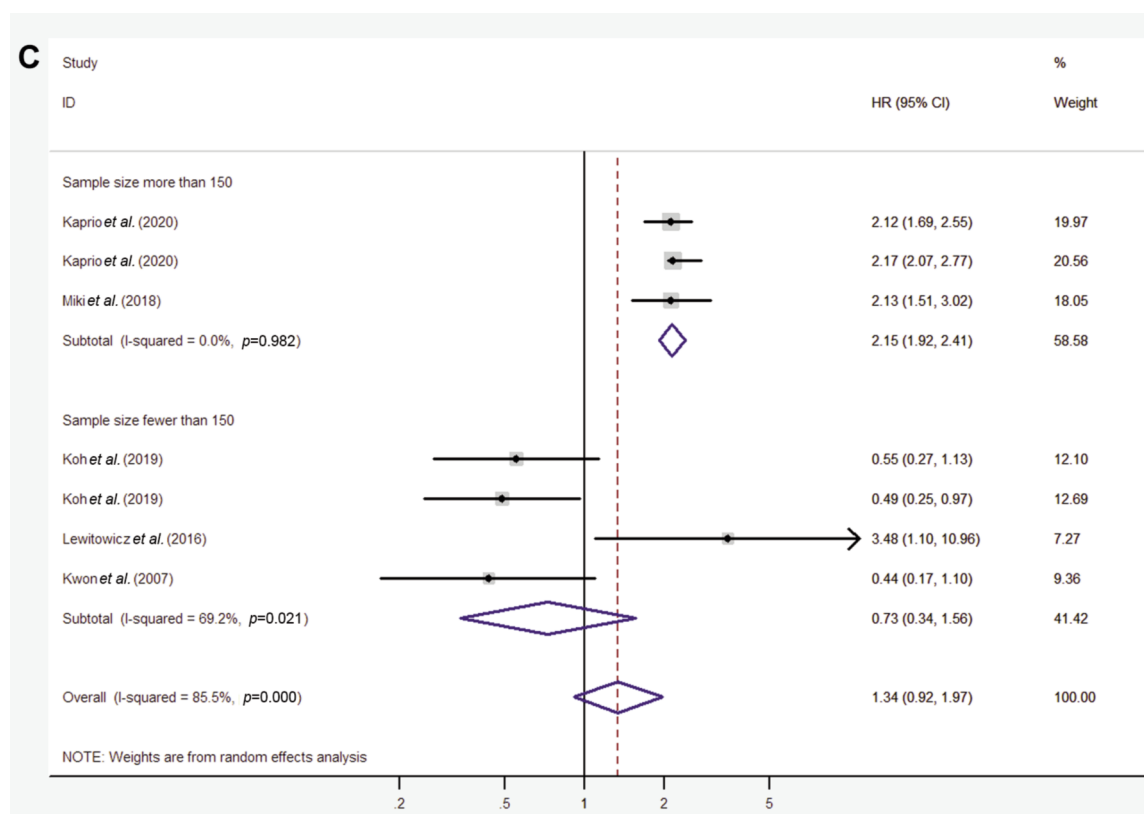


Figure 3. Forest plot for survival stratified by cancer type (A), survival outcome (B) and sample size (C).

The basic characteristics of included studies are summarized in Table I. The included studies consisted of the following cancer types: lung cancer (n=2), colorectal cancer (n=1), gastric cancer (n=1), and gastrointestinal stromal tumor (n=1). Two studies originated from Europe, three from Asia. The total number of patients included in this meta-analysis was 1,454, ranging from 54 to 595 per study. CD63 expression was evaluated by immunohistochemistry in all included studies. The NOS score of included studies was 7 or 8.

Association between CD63 expression and survival. The heterogeneity among the included studies was assessed using the random-effects model ($I^2=85.5%$, $p<0.001$). The HR evaluating CD63 expression on survival was 1.34 (95% CI=0.92-1.97, $p=0.129$) (Figure 2).

When grouped according to tumor type, the HRs of lung cancer and other tumors were 0.50 (95% CI=0.32-0.77, $p=0.002$) and 2.16 (95% CI=1.93-2.42, $p<0.001$) respectively (Figure 3A). Based on the survival outcomes, CD63 expression was significantly associated with poor disease-specific survival (HR=1.69, 95% CI=1.15-2.49, $p=0.008$), but not with disease-free survival and overall survival (Figure 3B). On the subgroup analysis of sample size, there

was a significant association between CD63 expression with poor survival in the group of sample size more than 150 (HR=2.15, 95% CI=2.92-2.41, $p<0.001$), but not in the group of sample size with fewer than 150 patients (Figure 3C).

Publication bias. As shown in Figure 4A, the Funnel plot showed an asymmetrical bias, but a significant publication bias was not identified (Egger's test, $p=0.075$). Therefore, the trim and filled analysis was performed. The analysis revealed that our results were unchanged (Figure 4B).

Sensitivity analysis. As shown in Figure 5, the sensitivity analysis showed that the two studies, by Kaprio *et al.* and Koh *et al.*, influenced our results. However, our initial results were still significant except for the effects of individual studies through sensitivity analysis, suggesting that our results are reliable (HR=1.97, 95% CI=1.77-2.20).

Discussion

CD63 is a member of tetraspanins that comprise a large superfamily of cell surface-associated membrane proteins and participate in various cell processes, like cell activation,

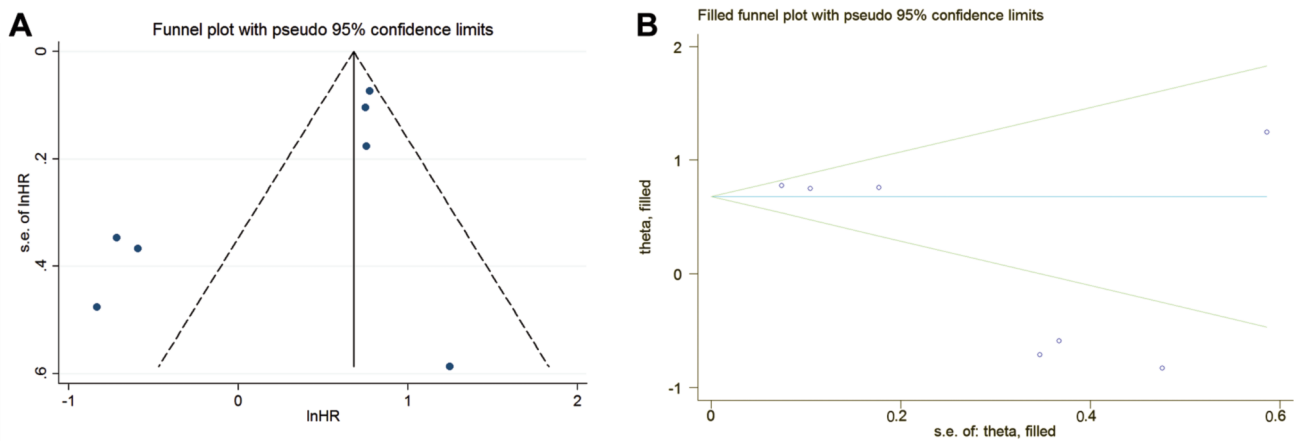


Figure 4. Funnel plot (A) and trim and fill method (B) for publication bias.

adhesion, differentiation, and tumor invasion (9). CD63 was firstly discovered as an abundantly expressed surface antigen in early-stage melanoma cells, and has been described as a tumor suppressor (9, 10). CD63 is associated with malignant progression of melanoma, wherein its expression decreases tumor invasion and metastasis by suppression of cellular motility and matrix-degrading ability in melanoma cells (7). A negative relationship between CD63 expression and increased malignancy is reported in various tumors, including ovarian, lung, breast, and colon cancer (5, 7, 9, 11-13).

On the contrary, it has recently become known that CD63 has also been implicated in the regulation of transport of other proteins involved in tumor development, invasiveness and metastasis (9). For example, Rank *et al.* have shown that CD63 levels are higher in the plasma of ovarian cancer patients compared to those with benign ovarian tumors (14). Moreover, CD63 may execute metastasis-promoting functions as a receptor of tissue inhibitor of metalloproteinases interacting with $\beta 1$ integrins (10). Furthermore, certain studies have revealed that CD63 expression is correlated with a tumor progression and lower survival (3, 6, 8, 15-17). Thus, we performed this meta-analysis to better understand the prognostic value of CD63 expression in solid tumors.

We identified 5 eligible studies with 1,454 patients for this meta-analysis. Kaprio *et al.*, Miki *et al.* and Lewitowicz *et al.* reported that positive or high expression of CD63 was associated with poor prognosis in colorectal cancer, gastric cancer and gastrointestinal stromal tumor respectively (3, 6, 8). On the other hand, Kown *et al.* and Koh *et al.* showed that negative or low expression of CD63 was correlated with a poor survival in lung adenocarcinoma and non-small cell lung cancer respectively (5, 7).

In the present study, we revealed that CD63 expression is associated with prognosis in patients with solid tumors (HR=1.34, 95% CI=0.92-1.97, $p=0.129$). In the subgroup

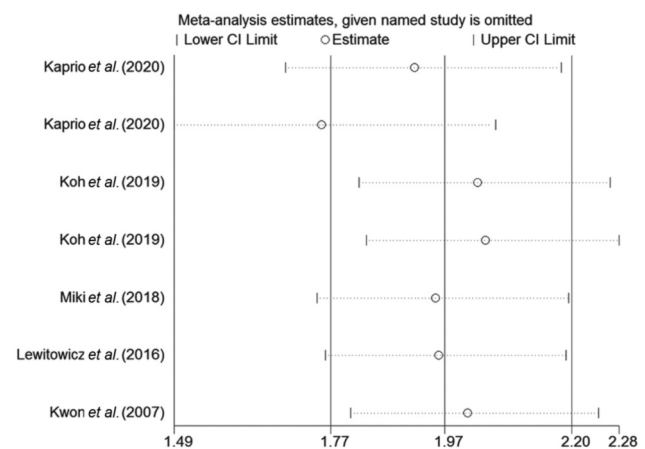


Figure 5. Sensitivity analysis of the association between CD63 expression and survival.

analysis, the HRs of lung cancer and other tumors were 0.50 (95% CI=0.32-0.77, $p=0.002$) and 2.16 (95% CI=1.93-2.42, $p<0.001$) respectively. The results suggested that CD63 expression may have a different effect on each tumor. Further research should reveal the effects of CD63 expression on the type of tumor. CD63 expression was significantly associated with disease-specific survival (HR=1.69, 95% CI=1.15-2.49, $p=0.008$) and sample size more than 150 (HR=2.15, 95% CI=2.92-2.41, $p<0.001$).

There are several limitations in the present study. Our results may be biased because of the small number of studies included. Therefore, further research requires a well-designed large-scale study. This analysis includes various studies examining the relationship between CD63 expression and prognosis in patients with tumors, so the heterogeneity between the studies cannot be blocked. We hope that future

studies will analyze the effects of CD63 expression on prognosis according to tumor type.

However, in the present study, we systematically evaluated the association between CD63 expression and prognosis in patients with solid tumors. To the best of our knowledge, this meta-analysis is the first report on the prognostic value of CD63 expression in solid tumors.

In summary, CD63 expression was associated with prognosis and might be a potential prognostic marker in solid tumors.

Conflicts of Interest

The Authors declare no potential conflicts of interest.

Authors' Contributions

H.M. Koh and D.C. Kim designed this review; H.M. Koh and B.G. Jang searched the databases and inspected all candidate articles; H.M. Koh and D.C. Kim extracted data and analyzed the data; H.M. Koh and B.G. Jang assessed the quality of included studies by the NOS; H.M. Koh wrote the manuscript, and all authors reviewed the manuscript.

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