Prognostic Value of Podoplanin in Various Tumors

Xiaohang Wang, PhD[1],*, Xueying Wang, MS[2],*,
Vladmir Carvalho, PhD[1], Qianqian Wang, MS[1], Tingting Li, MS[1],
Jinbang Wang, PhD[1], Yang Chen, PhD[1], Chengming Ni, PhD[1],
Subo Liu, BS[3], and Jiaxin Zhang, MS[2]

Abstract

Background: The prognostic significance of podoplanin (PDPN) in tumor cells for cancer patients' survival remains controversial. Therefore, we performed this meta-analysis to clarify the relationship between the podoplanin-positive tumor cells and cancer prognosis. **Method:** Eligible studies were identified by searching the Pubmed and EBSCO online databases up to August 2019. Hazard ratios (HRs) with 95% confidence intervals (CIs) were calculated to evaluate the correlation between podoplanin expression and overall survival (OS) and/or disease-free survival (DFS) and odds ratios (ORs) with 95% CIs severed as the summarized statistics for clinicopathological characteristic. **Results:** A total of 2155 patients from 21 eligible studies were included. The results revealed that high expression of podoplanin was associated with a poor survival rate in cancer patients. Further subgroup analysis stratified by tumor type showed that podoplanin-positive tumor cell infiltration had a negative prognostic effect associated with survival in esophageal cancer and oropharyngeal cancer. In addition, high expression of these cells was significantly associated with N stage, T stage, TNM stage and vascular invasion. **Conclusion:** Our study suggests the overexpression of podoplanin might be a significant prognostic indicator for patients with esophageal and oropharyngeal cancer.

Introduction

Cancer has become a heavy burden to the health care systems and is a leading cause of mortality worldwide.[1] Although diagnostic, treatment methods and supportive care have improved significantly over the past few decades, the cure rate of cancer is still relatively low. This is mainly due to the lack of screening strategies in the early stage, and the high recurrence rate of cancer. Targeted therapy early detection, and early treatment are efficient ways to prevent cancer recurrence. An increasing number of genetic targets are being used to suppress tumors. However, the sensitivity and specificity of most of the tumor markers remain controversial. Therefore, it is necessary to discover new genetic targets to prevent tumorigenesis and the development of cancer.

Podoplanin, a sialomucin-like type 1 transmembrane glycoprotein, also known as GP36,[2] GP40,[3] Aggrus, T1A,[4,5] has been found in a variety of normal tissues, including glomerular podocytes, lymphatic endothelial cells, heart, type I alveolar cells, and skeletal muscle.[6-8] Podoplanin plays a crucial role in cell motility, organ development, lymphangiogenesis, blood-lymph separation, platelet production, and immune response.[9,10] However, its physiological functions are still

[1] Department of Endocrinology, Zhongda Hospital, Institute of Diabetes, School of Medicine, Southeast University, Nanjing, China
[2] Department of Breast and Thyroid Surgery, Yangzhou University Affiliated Northern Jiangsu People's Hospital, Yangzhou, Jiangsu, China
[3] Department of Endocrinology, Shijiazhuang First Hospital, Shijiazhuang, China
* Xiaohang Wang and Xueying Wang contributed equally to this article

Corresponding Authors:
Jiaxin Zhang, MS, Department of Breast and Thyroid Surgery, Yangzhou University Affiliated Northern Jiangsu People's Hospital, Yangzhou, Jiangsu, China. Email: zhangjx62815@163.com

Subo Liu, BS, Department of Endocrinology, Shijiazhuang First Hospital, Shijiazhuang, China. Email: liusubo69@163.com

largely unknown. In pathology conditions, a growing body of evidence indicates that it plays an important role in tumorigenesis.[11-13] There is evidence that podoplanin correlates with tumor-associated lymphangiogenesis and cancer-associated fibroblasts (CAFs).[14-16] High expression of podoplanin has been observed in various kinds of tumor cells, such as oral cancer, esophageal cancer, lung cancer, cervical cancer, renal cancer, and cutaneous basal cell carcinoma.[17-19] However, the function of podoplanin remains controversial in tumor cells. In most kinds of cancers, the podoplanin expression is associated with a higher carcinoma cell migration, leading to cancer metastasis, lower survival rates, and poor tumor stage.[20-23] On the other hand, some studies suggest that podoplanin expression is a protective factor in lung and lip carcinoma.[24-26] Thus it is an important subject that needs to be further explored.

Therefore, we performed this meta-analysis to clarify the relationship between the density of podoplanin-positive tumor cells and outcomes such as overall survival (OS), disease-free survival (DFS), and prognosis factors in cancer patients.

Materials and Methods

Searching Strategy and Registration

We searched Pubmed and EBSCO websites to obtain the density of podoplanin in solid tumors and the survival in patients up to August 2019. The search strategy was: (podoplanin OR PDPN OR GP36 OR GP40 OR Aggrus OR T1A) AND (neoplasms [Title/Abstract] OR tumor [Title/Abstract] OR cancer [Title/Abstract] OR carcinoma [Title/Abstract]). The citation lists of the studies we searched were also used to identify other eligible studies. We completed the research registration on the INPLASY website (registration number: INPLASY202160113).

Inclusion and Exclusion Criteria

The studies were included in this meta-analysis when they met the following criteria: (1) Studies were published as original research articles. (2) The density of podoplanin was evaluated in human tissues. (3) They obtained the density of podoplanin^{+} tumor cells by histological or pathological examinations. (4) They provided hazard ratios (HRs) or odds ratios (ORs) with 95% confidence interval (CI), or Kaplan-Meier curves of high and low podoplanin^{+} tumor cells density with OS, and/or DFS.

The studies were excluded when they met the following criteria: (1) They were published as letters, case reports, reviews and conference abstracts, and in non-English languages. (2) Studies did not provide enough information to extract the data. (3) Studies detected podoplanin density not in tumor cells, or metastatic tissues. (4) Duplicate publications.

Data Extraction

All the information for this meta-analysis was extracted independently by 2 authors. The data we extracted were as follows: first author's name, publication year, number of patients, median age, gender, time of follow-up, tumor type, assessment method applied to quantify podoplanin^{+} tumor cells, and cut-off values of podoplanin expression levels. OS, DFS, and clinic pathological information including Tumor, Lymph Node, Metastasis (TNM) stage, lymphatic invasion were extracted from the text, tables, or Kaplan-Meier curves.

If the included studies showed the HRs and 95%CI in text or tables, we extracted it directly. Otherwise, we calculated the HRs and 95%CI from Kaplan-Meier curves via Engauge Digitizer version 4.1.

Quality Assessment

The quality of included studies was assessed by The Newcastle-Ottawa Scale (NOS). Two independent authors provided the NOS score and achieved consensus. A score ≥ 6 denoted a high quality.

Statistical Analysis

Statistical analysis was performed using STATA-MP software (Version 15.0, College Station, Texas, USA). Statistical heterogeneity was assessed with the Q-test or I^2 statistic were pooled based on the random-effect model in the presence of significant heterogeneity ($I^2 > 50\%$). Otherwise, the fixed-effect model was applied. Sensitivity analysis, Begg funnel plot, and Egger test were performed to assess the influence of individual study and the publication bias. All tests were 2-sided, and $P < 0.05$ was considered statistically significant.

Results

Search Results and Study Characteristics

The detailed flow diagram of the screening process is presented in Figure 1. A total of 8828 records were initially identified after searching on Pubmed and EBSCO. We then excluded duplicates and irrelevant studies after screening the titles and abstracts. Finally, we determined that 21 studies, including 2155 patients with solid tumors, met our inclusion criteria of this meta-analysis.[24-44] The quality of included studies was assessed by NOS, and the score ranged from 6 to 8. Characteristics of the included studies are shown in Table 1.

Meta-Analysis Results

Overall survival (OS). We combined extracted data into meta-analyses with STATA. The results have showed that podoplanin^{+} tumor cells were significantly associated with decreased OS (HR 1.739 95%CI 1.164 to 2.600, $P = 0.007$) in cancer patients (Figure 2).

The stratified analyses were implemented based on cancer type. As shown in Figure 3, pooled data indicated that podoplanin^{+} tumor cells were significantly associated with poor OS in esophageal carcinoma (HR 1.99, 95%CI 1.274 to 3.115, $P = 0.003$) and oropharyngeal carcinoma (HR 2.097, 95%CI 1.172 to 3.754, $P = 0.013$) with no heterogeneity detected ($I^2 = 0\%$, $P = 0.574$).
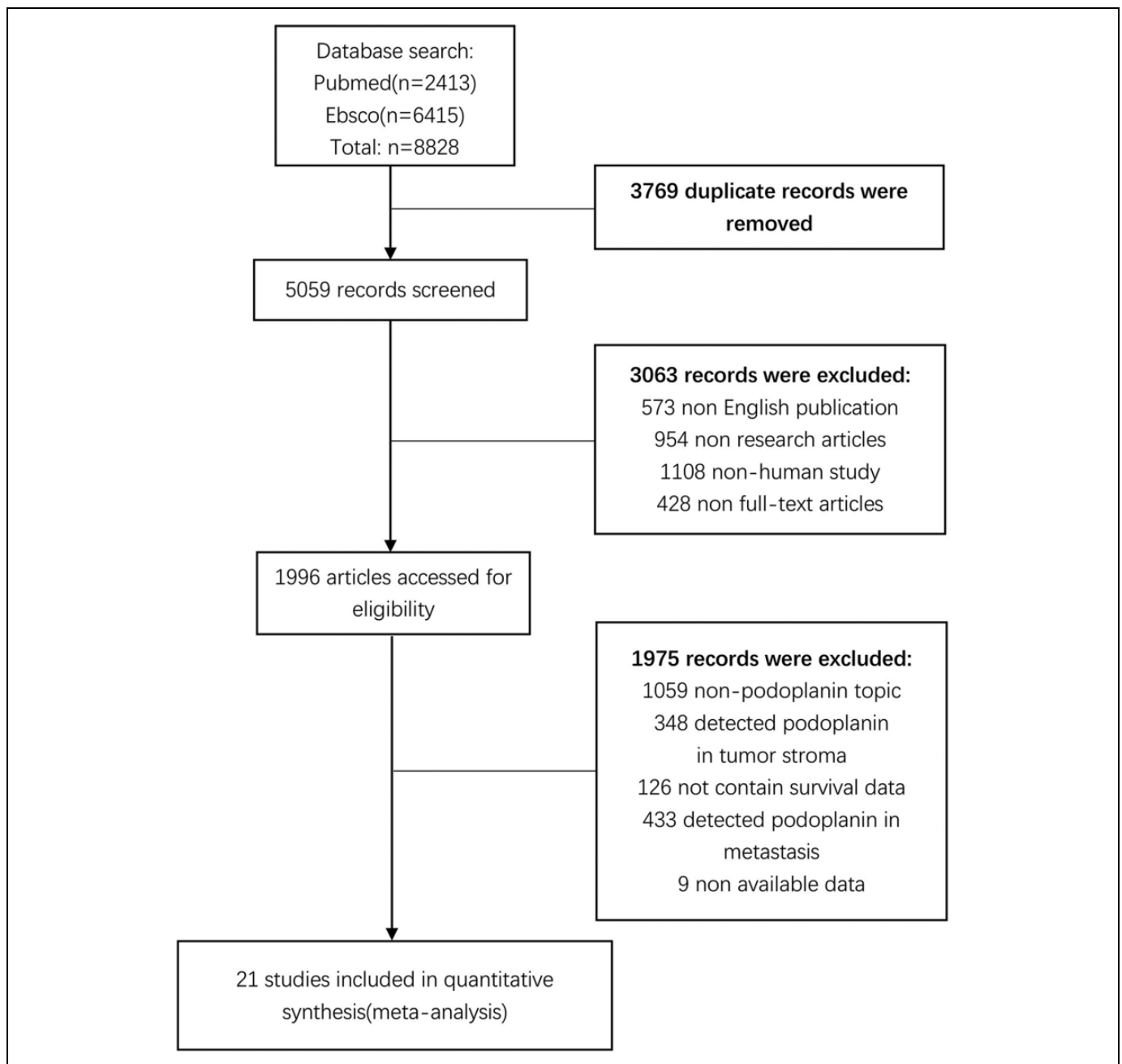
Figure 1. Flow chart of the data selection process. Finally, a total of 2155 patients from 21 eligible studies were included.

However, there was no significant association between podoplanin$^+$ tumor cells and OS in lung cancer (HR 0.697, 95%CI 0.324 to 1.499, P = 0.356) and head and neck carcinoma (HR 5.171, 95%CI 0.567 to 47.179, P = 0.145).

Disease-free survival (DFS). When we combined the DFS data into the meta-analysis, no significant relevance was observed in podoplanin$^+$ tumor cells with DFS (HR 1.496, 95%CI 0.961 to 2.329, P = 0.074) (Figure 4).

In stratified analysis by tumor type, as shown in Figure 5, podoplanin$^+$ tumor cells were significantly associated with poor DFS in esophageal carcinoma (HR 1.943, 95%CI 1.357

to 2.783, P < 0.001). And, conversely, elevated expression of podoplanin in tumors was not significantly associated with DFS in oropharyngeal carcinoma (HR 0.933, 95%CI 0.132 to 6.610, P = 0.944) and in lung carcinoma (HR 0.803, 95%CI 0.292 to 2.209, P = 0.672).

Association of Podoplanin Expression With Prognosis Factors

Moreover, we carried out meta-analyses with respect to prognostic characteristics. We found that high expression of podoplanin was significantly associated with poor N stage (N1,2 vs

Table 1. Characteristics of the Datasets Included in the Meta-Analysis of Association Between Podoplanin Expression and Solid Tumor.

| Study	Year	Tumor type	No of patients	Male/Female	Cut-offs	Pdpn+ cells H/L	Tumor stage	Survival	Median follow-up (Months)	Monoclonal antibody	NOS
Chao et al[30]	2012	ESCC	113	109/4	IRS $\geq$ 10	43/70	II-IV	5year-DFS/ 5year-DSS	NR	D2-40	6
Chuang et al[44]	2014	ESCC	59	58/1	IRS $\geq$ 10	25/34	I-IV	OS	NR	D2-40	6
Garcia et al[26]	2019	LSCC	91	75/16	IRS $\geq$ 10	42/49	I-IV	DFS	120	D2-40	7
Huttenlocher et al[42]	2014	SCCHN	160	124/36	>10%	58/102	I-IV	OS	24 (6-66)	D2-40	6
Ikoma et al[25]	2015	LSCC	103	97/6	$\geq$10%	32/71	NR	OS	52.4 (1.4-127.9)	D2-40	6
Ito et al[24]	2009	LSCC	136	117/19	>10%	69/67	IB	OS\DFS	44.5 (1.1-156.2)	D2-40	8
Juchniewicz et al[29]	2016	Esophageal cancer	84	76/8	qPCR	44/40	I-III	OS	25 (3-101)	NA	6
Kim et al[32]	2015	SCCHN	119	105/14	IRS $\geq$ 7	61/58	I-IV	DFS	40.6 (2-144)	NR	7
Kreppel et al[40]	2010	OSCC	80	51/29	score $\geq$ 2	49/31	I-IV	OS/DFS	42.9	D2-40	6
Kreppel et al[34]	2011	OSCC	63	48/15	score $\geq$ 2	38/25	I-IV	OS	90	D2-40	8
Kreppel et al[35]	2013	HNSCC	63	46/17	score $\geq$ 2	26/37	I-IV	OS	38	D2-40	8
Kubouchi et al[36]	2017	Lung adenocarcinoma	158	76/82	>10%	8/150	IA	OS/DFS	82.5 (8-151)	D2-40	6
Lin et al[28]	2017	Cervical cancer	130	0/130	>10%	61/69	Ib1-IIa2	OS/DFS	53.2 (18-89)	D2-40	6
Nakashima et al[33]	2013	ESCC	101	88/13	$\geq$20%	58/43	I-IV	OS	NR	D2-40	6
Rahadiani et al[27]	2010	ESCC	61	58/3	>10%	19/42	I-IV	OS/DFS	NR	D2-40	6
Saigusa et al[39]	2011	ESCC	20	18/2	>50%	12\8	I-IV	OS	20 (4-109)	D2-40	8
Tanaka et al[41]	2015	ESCC	139	128/11	$\geq$10%	47/92	I-IV	OS	NR	D2-40	8
Vinicius de et al[31]	2011	CSCC	55	32/23	>10%	23/32	I-III	DFS	25	D2-40	6
Xia et al[38]	2016	ccRCC	295	206/89	IOD score $\geq$ 220	176/119	I-IV	OS/DFS	98.97 (2.63-120.47)	18H5	8
Xie et al[43]	2018	LSCC	70	67/3	NR	28/42	I-IV	PFS (OS)	53.4	NR	6
Kan et al[37]	2014	Melanoma	55	21/34	$\geq$5%	35/20	I-IV	DFS	38.7 (2.6-90.1)	D2-40	7

Abbreviations: ESCC, esophageal squamous cell carcinoma; LSCC, lip squamous cell carcinoma; SCCHN, squamous cell carcinoma of the head and neck; OSCC, oral squamous cell carcinoma; CSCC, cutaneous squamous cell carcinoma; ccRCC, clear cell renal cell carcinoma; IRS, immunoreactive score; IOD score, integrated optical density score; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; NR, not reported.
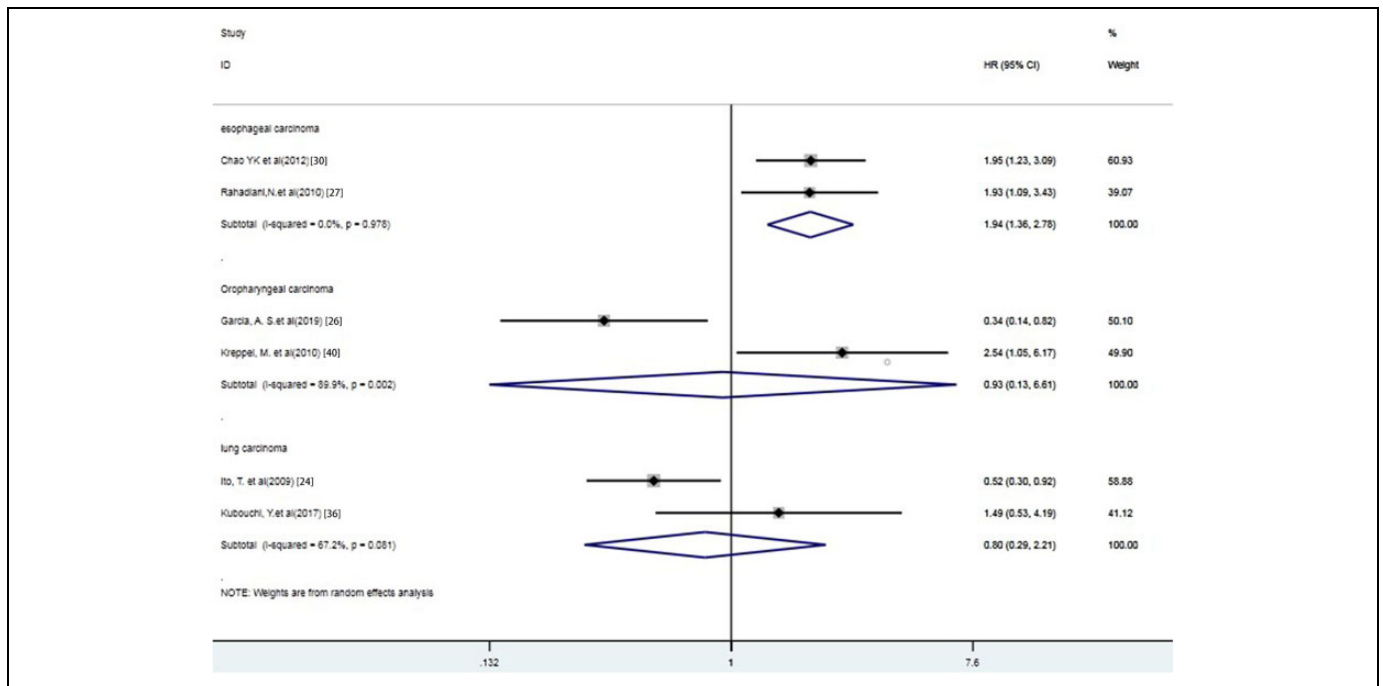
4
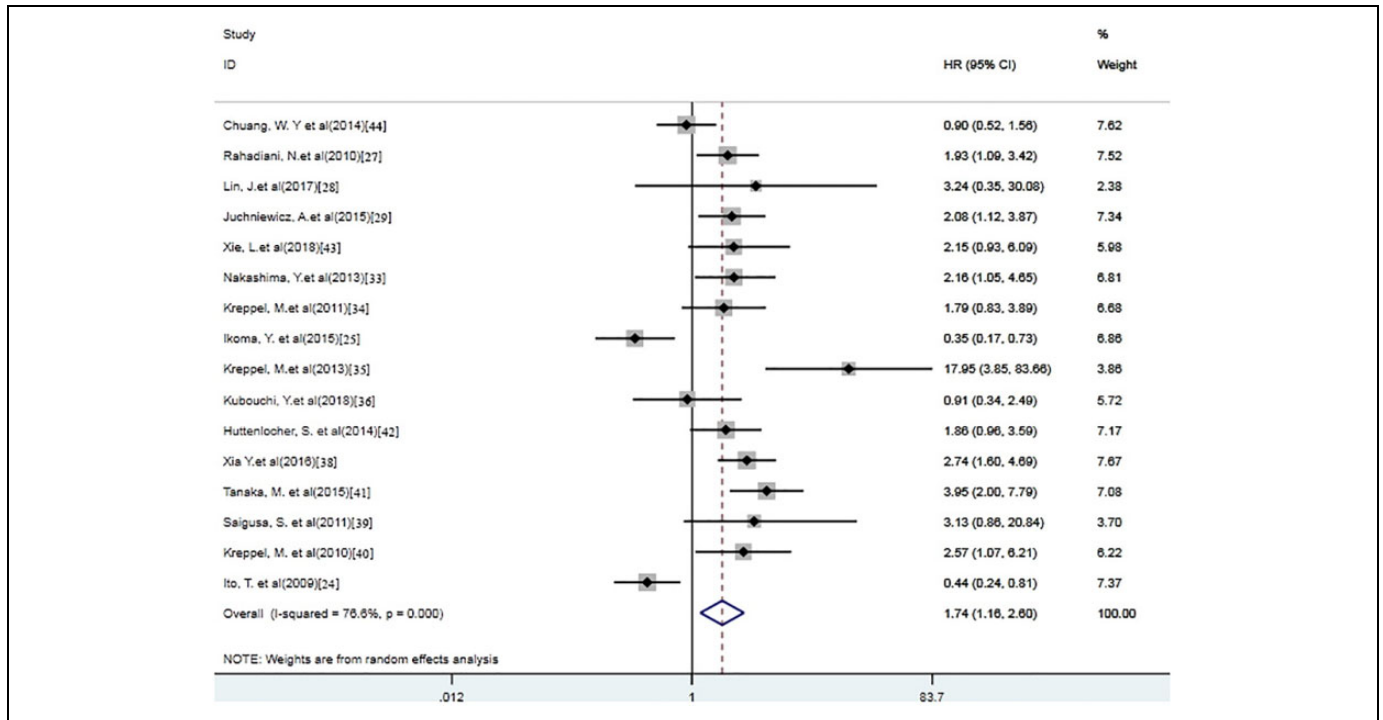
Figure 2. Forest plot describing HR of the association between podoplanin^{+} tumor cells and OS in tumors. Podoplanin^{+} tumor cells were significantly associated with decreased OS (HR 1.739, 95%CI 1.164 to 2.600, $P = 0.007$) in cancer patients.
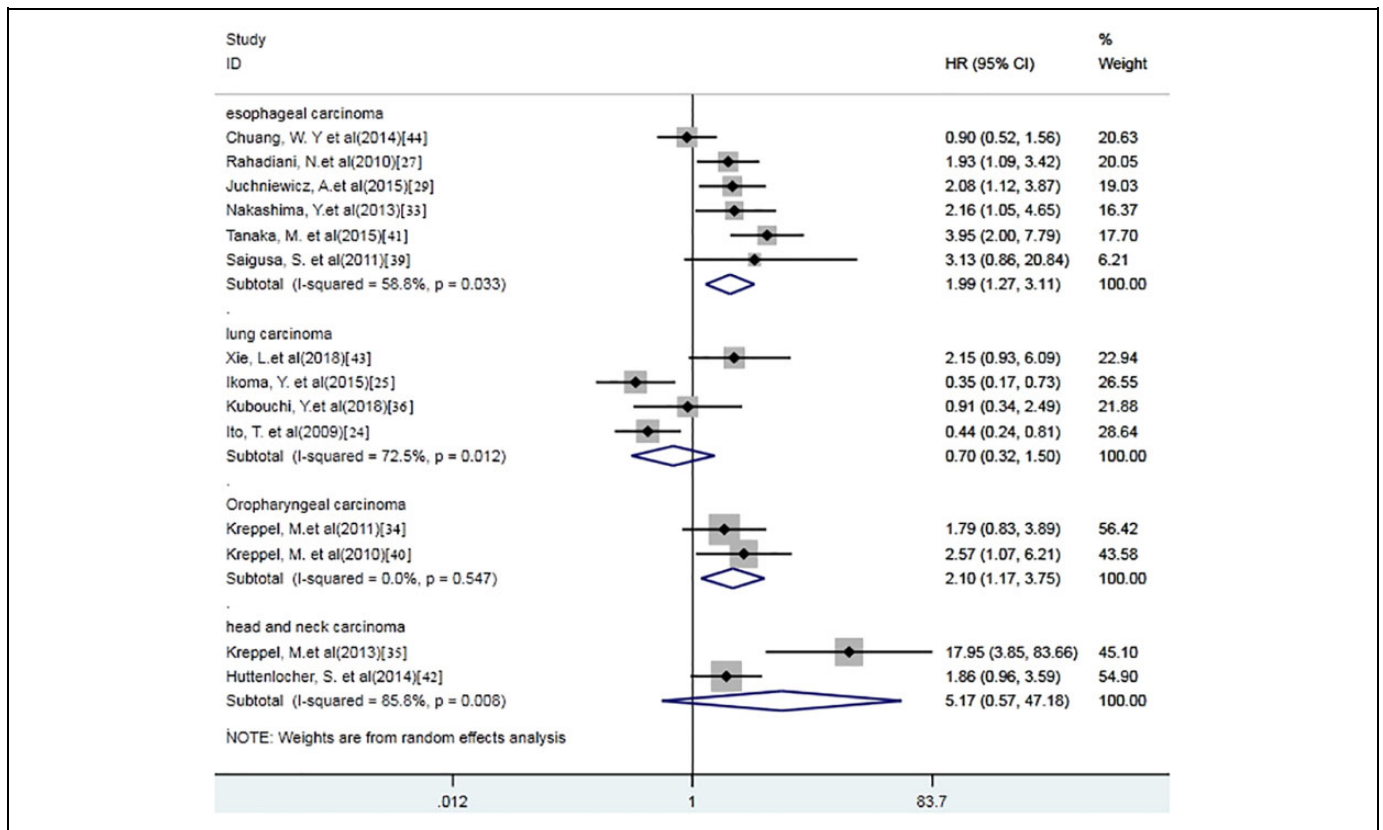
Figure 3. Stratified analyses describing HRs of the association between podoplanin^{+} tumor cells and OS. Podoplanin^{+} tumor cells were significantly associated with poor OS in esophageal carcinoma (HR 1.99, 95%CI 1.274 to 3.115, $P = 0.003$) and oropharyngeal carcinoma (HR 2.097, 95%CI 1.172 to 3.754, $P = 0.013$).
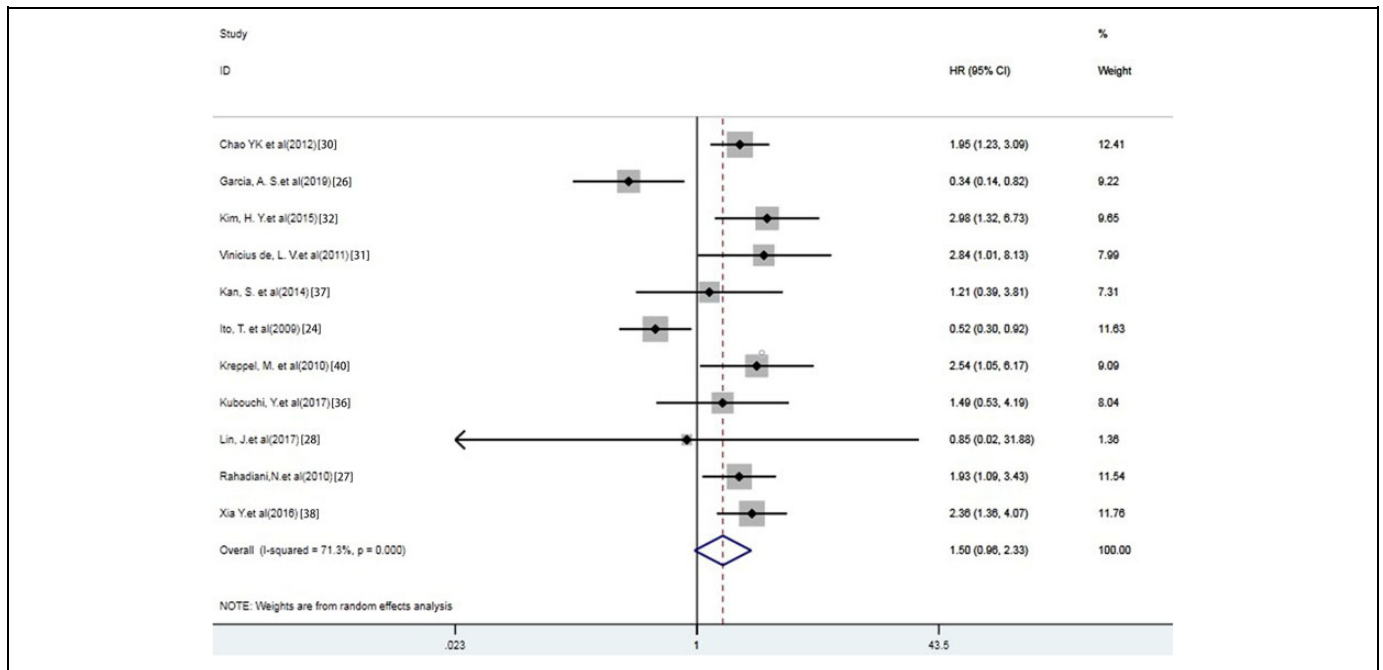
Figure 4. Forest plot describing HR of the association between podoplanin^{+} tumor cells and DFS in tumors. There was no significant relevance in podoplanin[+] tumor cell with DFS (HR 1.496, 95%CI 0.961 to 2.329, *P* = 0.074).

Figure 5. Stratified analyses describing HRs of the association between podoplanin^{+} tumor cells and DFS. Podoplanin^{+} tumor cells were significantly associated with poor DFS in esophageal carcinoma (HR 1.943, 95%CI 1.357 to 2.783, *P* < 0.001).

N0, OR = 3.652, 95%CI 2.366 to 5.636, *P* < 0.001), T stage (T3-4 vs T1-2, OR = 2.72, 95%CI 1.992 to 3.714, *P* < 0.001), TNM stage (III-IV vs I-II, OR = 3.083, 95%CI 2.141 to 4.439, *P* < 0.001) and vascular invasion (yes vs no, OR = 3.448, 95%CI 1.338 to 8.888, *P* = 0.010).

However, high podoplanin expression was not significantly related to age (≥60 vs <60, OR = 1.23, 95%CI 0.729 to 2.074, *P* = 0.438), gender (male vs female, OR = 1.113, 95%CI 0.768 to 1.611, *P* = 0.961), tumor size (≥3 vs <3cm, OR = 0.847, 95%CI 0.595 to 1.205, *P* = 0.355), Lymph node status (yes vs
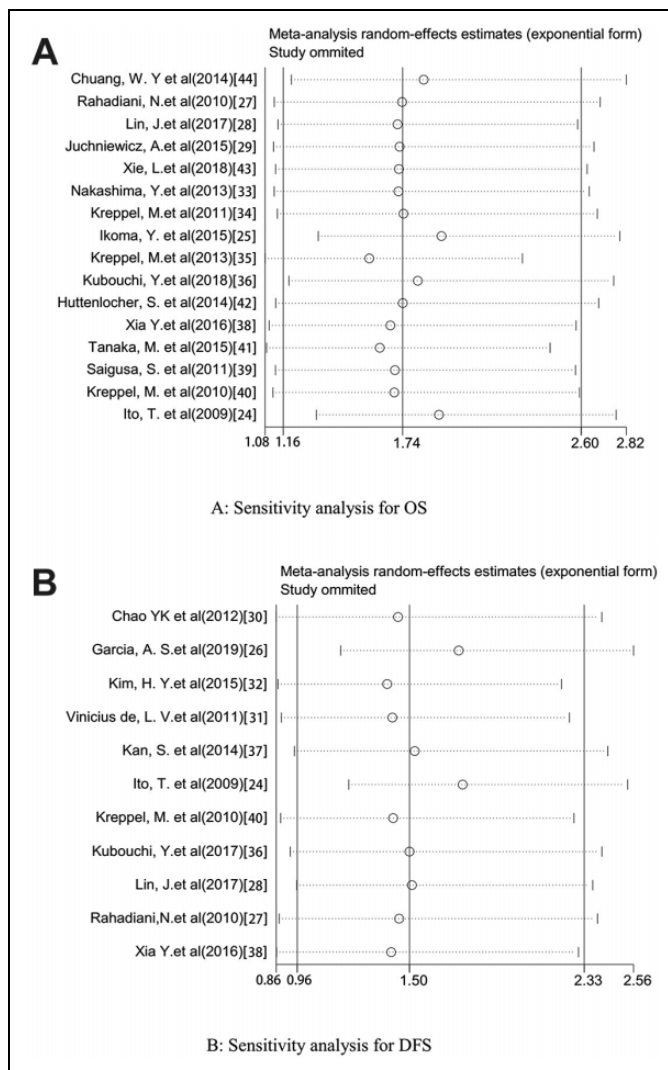
Figure 6. Plots describing the influence of individual studies on the overall HRs for OS (A) and DFS (B) in tumor patients. The results shown that each included study had no influence on the overall HR for OS or DFS.

no, OR = 1.826, 95%CI 0.881 to 3.784, P = 0.105), lymph vascular invasion (present vs absent, OR = 2.300, 95%CI 0.906 to 5.842, P = 0.080) and tumor differentiation (well vs poor, OR = 1.353, 95%CI 0.673 to 2.721, P = 0.396).

Sensitivity Analysis

Sensitivity analysis indicated that each included study had no influence on the overall HR for OS or DFS (Figure 6).

Publication Bias

The shape of the funnel plot and Egger's test did not reveal any publication bias existing between podoplanin-positive tumor cells and OS (P = 0.063) or DFS (P = 0.533).

Discussion

In recent years, the correlation between podoplanin expression and tumor prognosis has been explored in several studies, both in tumor stroma and tumor cells. However, the effect of podoplanin in tumor cells remains controversial. To further clarify the prognosis value of podoplanin in tumor cells, we performed this meta-analysis according to the Preferred Reporting Project (PRISMA) guideline.[45]

The present study involved 2155 patients from 21 eligible studies. Data from these studies revealed that high expression of podoplanin was associated with a poor survival rate in cancer patients. To determine the prognostic value of podoplanin in different cancers, we conducted stratified analyses by tumor types. The results showed that podoplanin-positive tumor cell infiltration had a negative prognostic effect associated with survival in esophageal cancer and oropharyngeal cancer. However, no relationship between podoplanin expression and survival was observed in other tumor-type patients. The results show a difference between OS and DFS. The reason for this discrepancy may be due to the limited number of eligible studies including DFS data, in which changes of some included studies could influence the pooled data. In addition, high expression of podoplanin in tumor cells was significantly associated with N stage, T stage, TNM stage and vascular invasion. No significant correlations were found with age, gender, tumor size, lymph node status, and tumor differentiation. These results provide meaningful evidence that the expression of podoplanin in tumor cells is a negative prognostic marker in esophageal cancer and oropharyngeal carcinoma. For such patients with high podoplanin expression in esophageal and oropharyngeal tumor cells, close follow-up is necessary. And targeting podoplanin is a new direction for cancer drug development.

Our findings regarding the adverse effects of podoplanin-positive tumor cells are consistent with those of prior studies.[11] The following reasons could possibly be responsible for the findings presented in this study. The extracellular region of podoplanin can bind to the C-type lectin-like receptor-2 (CLEC-2) receptor on platelets to induce platelet aggregation.[46,47] This interaction can promote tumor progression by releasing more cytokines like TGF-β and platelet-derived growth factor (PDGF) from the platelets and facilitate tumor-related thrombosis.[48] Some studies have shown that podoplanin localizes to the invadopodia and the invasive front of the tumor to facilitate tumor cell invasion and promote the progression of various types of cancer.[49] Podoplanin expression has been shown to enhance matrix metalloproteinase (MMP) expression, which degrades the extracellular matrix (ECM) during tumor invasion.[20] In addition, epithelial-mesenchymal transition (EMT) is a phenotypic change that manifests as epithelial cells lose polarity and epithelium markers such as E-cadherin, and obtaining migration factors such as snail and vimentin, a characteristic of fibroblasts.[50] This transition indicates a significant increase in tumor aggressiveness and metastasis. Several studies have

revealed that podoplanin plays an important role in the procession of EMT. High expression of podoplanin promotes EMT of tumor cells by releasing TGF-β from platelets.[51] Podoplanin can also promote vimentin expression in tumor cells, suggesting that it is involved in vimentin-associated EMT.[28]

In addition, the effect of podoplanin in tumor stroma was also worthy of study. Cremasco *et al* showed that podoplanin[+] CAFs exhibited immunosuppressive potential by releasing nitric oxide and then inhibited the proliferation of activated T cells.[52] Suzuki *et al* showed that podoplanin^{+} CAFs exhibited higher expression of TGFβ1 and were associated with CD204^{+} tumor-associated macrophages infiltration in stage-I lung squamous cell carcinoma.[13] Dai *et al* showed that the upregulation of podoplanin in CAFs increase tumor migration, proliferation, metastasis and malignancy.[53] Neri *et al* demonstrated that podoplanin[+] CAFs displayed a higher RhoA activity and treatment with a ROCK inhibitor canceled the increased invasion ability of podoplanin[+] CAFs and subsequently decreased the invasion and metastasis ability of tumor cells.[54] Hu *et al* performed a meta-analysis and found that podoplanin[+] CAFs infiltration had a negative prognostic effect associated with survival in breast cancer, lung cancer, pancreatic cancer and cholangiocarcinoma.[55] These findings all suggest that podoplanin[+] CAFs were associated with the immunosuppressive tumor microenvironment and tumor malignancy. Combined with our study, it is necessary to conduct a comprehensive systematic evaluation on the effect of podoplanin both in tumor and tumor stroma.

In this study, we made a comprehensive effort to present the clinical value of podoplanin expression in various cancer. However, several limitations should be considered when assessing the results from these analyses. First, non-English papers were excluded from this meta-analysis. Moreover, studies with negative results and low-quality may not have been published, which could lead to publication bias. However, there was no publication bias assessed by funnel plot and Egger's test in included studies. Second, some trials with negative results were missing relevant survival data, despite great efforts to contact the corresponding authors. Finally, podoplanin expression level was assessed via different concentrations of the podoplanin antibody, pathologists, and cut-off values, which may have led to some of the heterogeneity. Despite these limitations, the sensitivity analysis and Egger's test showed that these meta-analysis results were credible.

Conclusion

In conclusion, our study suggests that the over-expression of podoplanin might be a significant prognostic indicator for patients with esophageal and oropharyngeal cancer, implicating that it is a potential therapeutic target to improve survival in these cancer patients. However, more multi-center clinical investigations with larger sample sizes should be conducted to confirm these findings.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2020. *CA Cancer J Clin*. 2020;70(1):7-30. doi:10.3322/caac.21590
2. Zimmer G, Oeffner F, Von Messling V, et al. Cloning and characterization of gp36, a human mucin-type glycoprotein preferentially expressed in vascular endothelium. *Biochem J*. 1999;341(pt 2):277-284.
3. Zimmer G, Klenk HD, Herrler G. Identification of a 40-kDa cell surface sialoglycoprotein with the characteristics of a major influenza C virus receptor in a Madin-Darby canine kidney cell line. *J Biol Chem*. 1995;270(30):17815-17822. doi:10.1074/jbc.270.30.17815
4. Kato Y, Fujita N, Kunita A, et al. Molecular identification of Aggrus/T1alpha as a platelet aggregation-inducing factor expressed in colorectal tumors. *J Biol Chem*. 2003;278(51):51599-51605. doi:10.1074/jbc.M309935200
5. Rishi AK, Joyce-Brady M, Fisher J, et al. Cloning, characterization, and development expression of a rat lung alveolar type I cell gene in embryonic endodermal and neural derivatives. *Dev Biol*. 1995;167(1):294-306. doi:10.1006/dbio.1995.1024
6. Astarita JL, Cremasco V, Fu J, et al. The CLEC-2-podoplanin axis controls the contractility of fibroblastic reticular cells and lymph node microarchitecture. *Nat Immunol*. 2015;16(1):75-84. doi:10.1038/ni.3035
7. Quintanilla M, Montero-Montero L, Renart J, Martín-Villar E. Podoplanin in inflammation and cancer. *Int J Mol Sci*. 2019;20(3):707. doi:10.3390/ijms20030707
8. Krishnan H, Miller WT, Blanco FJ, Goldberg GS. Src and podoplanin forge a path to destruction. *Drug Discov Today*. 2019;24(1):241-249. doi:10.1016/j.drudis.2018.07.009
9. Acton SE, Astarita JL, Malhotra D, et al. Podoplanin-rich stromal networks induce dendritic cell motility via activation of the C-type lectin receptor CLEC-2. *Immunity*. 2012;37(2):276-289. doi:10.1016/j.immuni.2012.05.022

10. Herzog BH, Fu J, Wilson SJ, et al. Podoplanin maintains high endothelial venule integrity by interacting with platelet CLEC-2. *Nature*. 2013;502(7469):105-109. doi:10.1038/nature12501

11. Krishnan H, Rayes J, Miyashita T, et al. Podoplanin: an emerging cancer biomarker and therapeutic target. *Cancer Sci*. 2018;109(5):1292-1299. doi:10.1111/cas.13580

12. Zhou Q, Wang Z, Zeng H, et al. Identification and validation of poor prognosis immunoevasive subtype of muscle-invasive bladder cancer with tumor-infiltrating podoplanin(+) cell abundance. *Oncoimmunol*. 2020;9(1). doi:10.1080/2162402X.2020.1747333

13. Suzuki J, Aokage K, Neri S, et al. Relationship between podoplanin-expressing cancer-associated fibroblasts and the immune microenvironment of early lung squamous cell carcinoma. *Lung Cancer*. 2021;153:1-10. doi:10.1016/j.lungcan.2020.12.020

14. Bieniasz-Krzywiec P, Martín-Pérez R, Ehling M, et al. Podoplanin-expressing macrophages promote lymphangiogenesis and lymphoinvasion in breast cancer. *Cell Metab*. 2019;30(5):917-936.e10. doi:10.1016/j.cmet.2019.07.015

15. Yoshida T, Ishii G, Goto K, et al. Podoplanin-positive cancer-associated fibroblasts in the tumor microenvironment induce primary resistance to EGFR-TKIs in lung adenocarcinoma with EGFR mutation. *Clin Cancer Res*. 2015;21(3):642-651. doi:10.1158/1078-0432.ccr-14-0846

16. Nakamura H, Sugano M, Miyashita T, et al. Organoid culture containing cancer cells and stromal cells reveals that podoplanin-positive cancer-associated fibroblasts enhance proliferation of lung cancer cells. *Lung cancer (Amsterdam, Netherlands)*. 2019;134:100-107. doi:10.1016/j.lungcan.2019.04.007

17. Kawaguchi H, El-Naggar AK, Papadimitrakopoulou V, et al. Podoplanin: a novel marker for oral cancer risk in patients with oral premalignancy. *J Clin Oncol*. 2008;26(3):354-360. doi:10.1200/jco.2007.13.4072

18. Retzbach EP, Sheehan SA, Nevel EM, et al. Podoplanin emerges as a functionally relevant oral cancer biomarker and therapeutic target. *Oral Oncol*. 2018;78:126-136. doi:10.1016/j.oraloncology.2018.01.011

19. Chopra S, Deodhar K, Pai V, et al. Cancer stem cells, CD44, and outcomes following chemoradiation in locally advanced cervical cancer: results from a prospective study. *Int J Radiat Oncol Biol Phys*. 2019;103(1):161-168. doi:10.1016/j.ijrobp.2018.09.003

20. Monteiro LS, Delgado ML, Ricardo S, et al. Prognostic significance of CD44v6, p63, podoplanin and MMP-9 in oral squamous cell carcinomas. *Oral Dis*. 2016;22(4):303-312. doi:10.1111/odi.12442

21. Bartuli FN, Luciani F, Caddeo F, et al. Podoplanin in the development and progression of oral cavity cancer: a preliminary study. *Oral Implantol (Rome)*. 2012;5(2-3):33-41.

22. Barros FBA, Assao A, Garcia NG, et al. Moesin expression by tumor cells is an unfavorable prognostic biomarker for oral cancer. *BMC Cancer*. 2018;18(1):53. doi:10.1186/s12885-017-3914-0

23. Ugorski M, Dziegiel P, Suchanski J. Podoplanin—a small glycoprotein with many faces. *Am J Cancer Res*. 2016;6(2):370-386.

24. Ito T, Ishii G, Nagai K, et al. Low podoplanin expression of tumor cells predicts poor prognosis in pathological stage IB squamous cell carcinoma of the lung, tissue microarray analysis of 136 patients using 24 antibodies. *Lung Cancer*. 2009;63(3):418-424. doi:10.1016/j.lungcan.2008.06.008

25. Ikoma Y, Kijima H, Masuda R, Tanaka M, Inokuchi S, Iwazaki M. Podoplanin expression is correlated with the prognosis of lung squamous cell carcinoma. *Biomed Res (Tokyo, Japan)*. 2015;36(6):393-402. doi:10.2220/biomedres.36.393

26. Garcia AS, Assao A, Carvalho AL, Soares FA, Kowalski LP, Oliveira DT. The stem cell markers expression CD44v6 and podoplanin in lip cancer: clinical significance. *Virchows Arch*. 2019;474(6):745-754. doi:10.1007/s00428-019-02539-3

27. Rahadiani N, Ikeda J, Makino T, et al. Tumorigenic role of podoplanin in esophageal squamous-cell carcinoma. *Ann Surg Oncol*. 2010;17(5):1311-1323. doi:10.1245/s10434-009-0895-5

28. Lin J, Lu J, Wang C, Xue X. The prognostic values of the expression of vimentin, TP53, and podoplanin in patients with cervical cancer. *Cancer Cell Int*. 2017;17:80. doi:10.1186/s12935-017-0450-6

29. Juchniewicz A, Niklinska W, Kowalczuk O, et al. Prognostic value of vascular endothelial growth factor-C and podoplanin mRNA expression in esophageal cancer. *Oncol Lett*. 2015;10(6):3668-3674. doi:10.3892/ol.2015.3824

30. Chao YK, Chuang WY, Yeh CJ, et al. Prognostic significance of high podoplanin expression after chemoradiotherapy in esophageal squamous cell carcinoma patients. *J Surg Oncol*. 2012;105(2):183-188. doi:10.1002/jso.22068

31. Vinicius de LV, Scapulatempo C, Perpetuo NM, et al. Prognostic and risk factors in patients with locally advanced cutaneous squamous cell carcinoma of the trunk and extremities. *J Skin Cancer*. 2011;2011. doi:10.1155/2011/420796

32. Kim HY, Rha KS, Shim GA, et al. Podoplanin is involved in the prognosis of head and neck squamous cell carcinoma through interaction with VEGF-C. *Oncol Rep*. 2015;34(2):833-842. doi:10.3892/or.2015.4070

33. Nakashima Y, Yoshinaga K, Kitao H, et al. Podoplanin is expressed at the invasive front of esophageal squamous cell carcinomas and is involved in collective cell invasion. *Cancer Sci*. 2013;104(12):1718-1725. doi:10.1111/cas.12286

34. Kreppel M, Drebber U, Wedemeyer I, et al. Podoplanin expression predicts prognosis in patients with oral squamous cell carcinoma treated with neoadjuvant radiochemotherapy. *Oral Oncol*. 2011;47(9):873-878. doi:10.1016/j.oraloncology.2011.06.508

35. Kreppel M, Krakowezki A, Kreppel B, et al. Podoplanin expression in cutaneous head and neck squamous cell carcinoma—prognostic value and clinicopathologic implications. *J Surg Oncol*. 2013;107(4):376-383. doi:10.1002/jso.23238

36. Kubouchi Y, Yurugi Y, Wakahara M, et al. Podoplanin expression in cancer-associated fibroblasts predicts unfavourable prognosis in patients with pathological stage IA lung adenocarcinoma. *Histopathology*. 2018;72(3):490-499. doi:10.1111/his.13390

37. Kan S, Konishi E, Arita T, et al. Podoplanin expression in cancer-associated fibroblasts predicts aggressive behavior in melanoma. *J Cutan Pathol*. 2014;41(7):561-567. doi:10.1111/cup.12322

38. Xia Y, Liu L, Xiong Y, et al. Podoplanin associates with adverse postoperative prognosis of patients with clear cell renal cell carcinoma. *Cancer Sci*. 2016;107(9):1243-1249. doi:10.1111/cas.13007

39. Saigusa S, Mohri Y, Ohi M, et al. Podoplanin and SOX2 expression in esophageal squamous cell carcinoma after neoadjuvant chemo-radiotherapy. *Oncol Rep*. 2011;26(5):1069-1074. doi:10.3892/or.2011.1408

40. Kreppel M, Scheer M, Drebber U, Ritter L, Zoller JE. Impact of podoplanin expression in oral squamous cell carcinoma: clinical and histopathologic correlations. *Virchows Arch*. 2010;456(5): 473-482. doi:10.1007/s00428-010-0915-7

41. Tanaka M, Kijima H, Shimada H, Makuuchi H, Ozawa S, Inokuchi S. Expression of podoplanin and vimentin is correlated with prognosis in esophageal squamous cell carcinoma. *Mol Med Rep*. 2015;12(3):4029-4036. doi:10.3892/mmr.2015.3966

42. Huttenlocher S, Seibold ND, Gebhard MP, et al. Evaluation of the prognostic role of tumor cell podoplanin expression in locally advanced squamous cell carcinoma of the head and neck. *Strahlenther Onkol*. 2014;190(11):1021-1027. doi:10.1007/s00066-014-0694-1

43. Xie L, Lin C, Zhang Q, et al. Elevated expression of podoplanin and its clinicopathological, prognostic, and therapeutic values in squamous non-small cell lung cancer. *Cancer Manag Res*. 2018; 10:1329-1340. doi:10.2147/cmar.s163510

44. Chuang WY, Yeh CJ, Chao YK, et al. Concordant podoplanin expression in cancer-associated fibroblasts and tumor cells is an adverse prognostic factor in esophageal squamous cell carcinoma. *Int J Clin Exp Pathol*. 2014;7(8):4847-4856.

45. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Ann Intern Med*. 2009;151(4): 264-269, W64. doi:10.7326/0003-4819-151-4-200908180-00135

46. Kato Y, Kaneko MK, Kunita A, et al. Molecular analysis of the pathophysiological binding of the platelet aggregation-inducing factor podoplanin to the C-type lectin-like receptor CLEC-2. *Cancer Sci*. 2008;99(1):54-61. doi:10.1111/j.1349-7006.2007.00634.x

47. Suzuki-Inoue K, Kato Y, Inoue O, et al. Involvement of the snake toxin receptor CLEC-2, in podoplanin-mediated platelet activation, by cancer cells. *J Biol Chem*. 2007;282(36):25993-26001. doi:10.1074/jbc.M702327200

48. Suzuki-Inoue K. Platelets and cancer-associated thrombosis: focusing on the platelet activation receptor CLEC-2 and podoplanin. *Blood*. 2019;134(22):1912-1918. doi:10.1182/blood.2019001388

49. Wicki A, lehembre F, Wick N, Hantusch B, Kerjaschki D, Christofori G. Tumor invasion in the absence of epithelial-mesenchymal transition: podoplanin-mediated remodeling of the actin cytoskeleton. *Cancer Cell*. 2006;9(4):261-272. doi:10.1016/j.ccr.2006.03.010

50. Dongre A, Weinberg RA. New insights into the mechanisms of epithelial-mesenchymal transition and implications for cancer. *Nat Rev Mol Cell Biol*. 2019;20(2):69-84. doi:10.1038/s41580-018-0080-4

51. Takemoto A, Okitaka M, Takagi S, et al. A critical role of platelet TGF-β release in podoplanin-mediated tumour invasion and metastasis. *Sci Rep*. 2017;7. doi:10.1038/srep42186

52. Cremasco V, Astarita JL, Grauel AL, et al. FAP delineates heterogeneous and functionally divergent stromal cells in immune-excluded breast tumors. *Cancer Immunol Res*. 2018;6(12): 1472-1485. doi:10.1158/2326-6066.CIR-18-0098

53. Dai K, Tanaka M, Kamiyoshi A, et al. Deficiency of the adrenomedullin-RAMP3 system suppresses metastasis through the modification of cancer-associated fibroblasts. *Oncogene*. 2020;39(9):1914-1930. doi:10.1038/s41388-019-1112-z

54. Neri S, Ishii G, Hashimoto H, et al. Podoplanin-expressing cancer-associated fibroblasts lead and enhance the local invasion of cancer cells in lung adenocarcinoma. *Int J Cancer*. 2015; 137(4):784-796. doi:10.1002/ijc.29464

55. Hu G, Wang S, Xu F, et al. Tumor-infiltrating podoplanin+ fibroblasts predict worse outcome in solid tumors. *Cell Physiol Biochem*. 2018;51(3):1041-1050. doi:10.1159/000495484