Original Article

# CD276 as a critical independent biomarker and immune checkpoint inhibitor target in epithelioid mesothelioma-TCGA study

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**Background:** CD276 is an immune checkpoint, and immune checkpoint inhibitors (ICIs) targeting CD276 have been tested against various cancers. However, the precise role of CD276 in mesothelioma subtypes is unknown. This study aimed to reveal the prognostic significance of CD276 in various cancers and explore CD276 as a target for ICIs in different mesothelioma subtypes.

**Methods:** We evaluated data from The Cancer Genome Atlas (TCGA) database retrospectively. The Wilcoxon rank-sum test was used to assess *CD276* mRNA expression between cancer tissues and the adjacent normal tissues in the context of various cancers. The study involved 86 patients with mesothelioma. The mean number of patients was set as the cutoff value for comparing *CD276* mRNA expression. The overall survival (OS) of patients with each mesothelioma subtype was estimated using the Kaplan-Meier method with *CD276* mRNA expression. The factors affecting the correlation between OS and high/low *CD276* expression in combination with/without a current existing molecular targets of programmed cell death 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA4), and vascular endothelial growth factor A (VEGFA) were assessed using a multivariate Cox proportional hazards model. The correlation between the mRNA expression of *CD276* and expression of gene markers of tumor-infiltrating immune cells and those of different pathways was evaluated using Spearman's correlation. The factors affecting correlations of *CD276* mRNA expression were confirmed using a multivariate linear regression model.

**Results:** Upregulated *CD276* mRNA expression was associated with a poor prognosis in various cancers, including epithelioid mesothelioma. The multivariate Cox proportional hazards model demonstrated that upregulated *CD276* mRNA expression indicated the worst prognosis, including the combination of *CD276* and PD-1, *CTLA4*, and *VEGFA*. In addition, using a multivariate linear regression model, *CD276* mRNA expression was found to correlate with multiple glycolytic pathway mRNAs in epithelioid mesothelioma, especially *PKM2*.

**Conclusions:** CD276 is an independent prognostic biomarker in patients with epithelioid mesothelioma. It is associated with the glycolytic pathway and may contribute to ATP generation in epithelioid mesothelioma. CD276 inhibitors might contribute to better prognosis in patients with epithelioid mesothelioma.

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**Keywords:** B7-H3/CD276; epithelioid mesothelioma; immune checkpoint inhibitor (ICI); novel prognostic biomarker; The Cancer Genome Atlas (TCGA)

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## Introduction

### *Background*

Mesothelioma is a rare and refractory cancer (1,2). Its tumorigenesis is related to the exposure (3,4) of mesothelial cells (5) to asbestos following a latency period of 30–40 years (6,7). However, the mechanisms underlying mesothelioma tumorigenesis are not fully understood (3,5). Although some molecular targets and possible prognostic biomarkers for mesothelioma have been identified (8-10), they have been inconclusive. One reason for this inconclusiveness is that, despite the identification of tumor suppressor genes, mesothelioma-related oncogenes are not well understood (11-13). A combination of immune checkpoint inhibitors (ICIs), namely nivolumab and ipilimumab, which are programmed cell death 1 (PD-1) and cytotoxic T-lymphocyte-associated protein 4 (CTLA4) inhibitors (14), and chemotherapy with cisplatin and pemetrexed (15) with/without bevacizumab, which is a vascular endothelial growth factor A (VEGFA) inhibitor (16), have been confirmed to be effective in a phase 3 study of mesothelioma. However, the overall survival (OS) of patients administered these treatments was 12–18 months, with many patients eventually developing resistance to treatment (14,15). Mesothelioma consists of epithelioid and nonepithelioid subtypes, including biphasic and sarcomatoid subtypes. The prognosis and treatment-associated OS vary according to the subtype (2,3,5-11,13,14).

Although ICIs such as nivolumab and ipilimumab have been demonstrated to be effective against nonepithelioid mesothelioma, their efficacy against epithelioid mesothelioma is unclear, and prognostic biomarkers are lacking (14). Identifying novel molecular targets and prognostic biomarkers for each subtype is essential for the personalized treatment of patients with mesothelioma. In a recent study, we demonstrated that ICIs targeting lymphocyte-activation gene 3 (LAG3) and PD-1 inhibitors could potentially contribute to better clinical outcomes, and LAG3 expression was identified as an independent prognostic biomarker for mesothelioma (17). With the development of novel ICIs, there has been improvement in cancer treatment outcomes (18). The B7 homolog 3 (B7-H3) protein encoded by *CD276* was first reported in 2001; it is a member of the B7 family and a key immune checkpoint that is highly expressed on the membrane of normal and cancer cells (19). The structure of B7-H3 comprises an IgV domain, an IgC-like domain, a transmembrane region, and a diverse cytoplasmic tail (20). Although the receptor of B7-H3 has not been confirmed, B7-H3/CD276 is considered to play a key role in the escape of cancer cells from the immune system through the production of IL-10 and TGF-β1 (20). B7-H3/CD276 inhibits the proliferation, activity, and function of CD4/CD8+ T-cells, natural killer cells, macrophages, and dendritic cells and stimulates the function of regulatory T-cells (20,21). In addition, B7-H3/CD276 has been suggested to be associated with metabolic reprogramming via the glycolytic pathway (22), supporting the notion of the Warburg effect (23). It is also reportedly associated with the ferroptosis pathway that is correlated with cancer cell proliferation (24); angiogenesis via the NF-κB pathway (25); and the proliferation, migration, and invasion of cells via the JAK/STAT3, PIK3CA/AKT/mTOR

### Highlight box

**Key findings**

- CD276 is an independent prognostic biomarker and immune checkpoint inhibitor (ICI) target in epithelioid mesothelioma. CD276 is associated with the glycolytic pathway and may be involved in ATP generation in epithelioid mesothelioma.

**What is known and what is new?**

- ICIs targeting CD276 have been tested against various cancers. However, the specific role of CD276 in mesothelioma subtypes has remained unknown.
- Here, we explored the prognostic significance of CD276 and its potential as a target for ICIs in different mesothelioma subtypes.

**What is the implication and what should change now?**

- CD276 is a target for ICIs in patients with epithelioid mesothelioma; CD276 inhibitors should be studied further for their potential in improving prognosis in patients with epithelioid mesothelioma.

and RAF/MEK pathways (26-28).

### *Rationale and knowledge gap*

High expression of CD276 is correlated with a poor prognosis in various cancers (29,30), including mesothelioma. However, mesothelioma subtypes and multivariate Cox proportional hazards models were not considered in these analyses (31,32). Furthermore, CD276 inhibitors downregulated cancer cell proliferation in an *in vivo* study (33), and various clinical studies on CD276 inhibitors are currently ongoing (30).

### *Objective*

While the exact significance of CD276 remains unknown in various cancer, particularly in each mesothelioma subtype, we hypothesize that CD276 could be a potentially novel biomarker and the therapeutic target for each mesothelioma subtype. Therefore, this study aimed to evaluate *CD276* mRNA expression as a prognostic biomarker across various cancers and explore its potential as a therapeutic target for each mesothelioma subtype, considering the existing therapeutic strategies for mesothelioma using molecular targets of PD-1 (*PDCD1*), *CTLA4*, and *VEGFA*. Our study could provide a new perspective on the correlation between *CD276* and various associated pathways in each mesothelioma subtype. We present this article in accordance with the REMARK reporting checklist (available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1598/rc).

## Methods

### *The Cancer Genome Atlas (TCGA) database*

We extracted data from TCGA database, which is a cancer genomics project started by the National Cancer Institute in 2006, and evaluated the data retrospectively. *CD276* mRNA expression between 17 types of cancer and normal tissues was analyzed using TCGA (https://cistrome.shinyapps.io/timer/), as previously described (17,34). To compare *CD276* expression between cancer and the adjacent normal tissues, the mRNA expression was transformed to log2 transcripts per million (17,34). The *CD276* mRNA expression and OS data of 36 human cancer types were analyzed using TCGA database (https://xena.ucsc.edu/), as previously described (17,34). To compare high and low *CD276* expression in TCGA, the mean number of patients was used as a cutoff value. Number of patients, age, sex, disease stage, and histological evaluation from TCGA were used as clinical variables for assessing factors affecting the correlation between OS and high/low expression of *CD276*, *PDCD1*, *CTLA4*, and *VEGFA* mRNA, and the combinations of high/low expression of *CD276* and *PDCD1*, *CTLA4*, *VEGFA*, and clinical variables (17). The correlation of *CD276* mRNA expression with the expression of gene markers of tumor-infiltrating immune cells (TIICs) and the glycolytic, ferroptosis, NF-κB, JAK/STAT3, PIK3CA/AKT/mTOR, and RAF/MEK pathways in TCGA was also evaluated.

### *Statistical analysis*

All statistical analyses were conducted using R 3.6.2 (The R Foundation for Statistical Computing, Vienna, Austria) and GraphPad PRISM 10.3 (GraphPad Software, La Jolla, CA, USA) (17,34). Comparison of mRNA expression between cancer and the adjacent normal tissues was performed using the Wilcoxon rank-sum test (17,34). The correlation of high/low *CD276* mRNA expression with OS was evaluated via the Kaplan-Meier method using the log-rank test with the hazard ratio (HR) and 95% confidence interval (CI) (17,34). A multivariate Cox proportional hazards model adjusted for age, sex, and disease stage as basic data was used to evaluate factors affecting the correlation between OS and high/low *CD276* mRNA expression (17,34).

The correlation between the mRNA expression of *CD276* and expression of gene markers of TIICs and those of glycolytic, ferroptosis, NF-κB, JAK/STAT3, PIK3CA/AKT/mTOR, and RAF/MEK pathways was evaluated using the Spearman's rank correlation coefficient using r (17). The r values estimated using the 95% CI indicated the following: 0.80–1.0, very strong correlation; 0.60–0.79, strong; 0.40–0.59, moderate; 0.20–0.39, weak; and 0.001–0.19, very weak (17). A multivariate linear regression model was used to confirm the factors affecting the correlation of *CD276* mRNA expression using β value, which is the regression coefficient with a 95% CI. Results were considered statistically significant at P value <0.05 (17).

### *Ethical statement*

We used data from an anonymized public open database, which was outside the scope of the Japanese ethical guidelines and was thus exempt from ethical scrutiny. Obtaining patient consent was waived by the Institutional Review Board of Tokyo Women's Medical University for

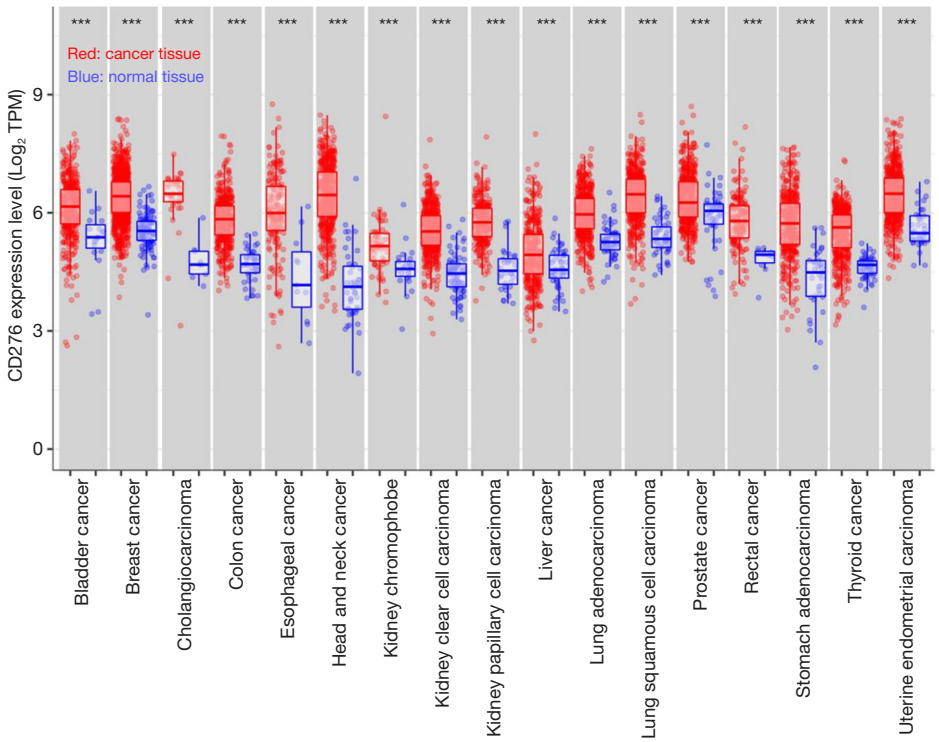


**Figure 1** Comparison of *CD276* mRNA expression between cancer tissues and the adjacent normal tissues in various human cancers. Comparison of *CD276* mRNA expression between cancer tissues and normal tissues in various human cancers was performed using TIMER (http://cistrome.org/TIMER/) and visualized using the Wilcoxon rank-sum test. *CD276* mRNA expression in all cancer tissues, including bladder cancer, breast cancer, cholangiocarcinoma, colon cancer, esophageal cancer, head and neck cancer, kidney chromophobe, kidney clear cell carcinoma, kidney papillary cell carcinoma, liver cancer, lung adenocarcinoma, lung squamous cell carcinoma, prostate cancer, rectal cancer, thyroid cancer, and uterine endometrial carcinoma, was higher than that in normal tissues (***, P<0.001). Red: cancer tissues; blue: normal tissues. TPM, transcripts per million.

this retrospective study. This manuscript does not include data from any human or animal studies conducted by the authors. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki (as revised in 2013).

## Results

### *Comparison of CD276 mRNA expression between cancer and adjacent normal tissues in human cancers*

To determine *CD276* mRNA expression in cancer, we compared *CD276* mRNA expression between cancer tissues and adjacent normal tissues in various human cancers. We found that *CD276* mRNA expression was higher in all cancers, including bladder cancer, breast cancer, cholangiocarcinoma, colon cancer, esophageal cancer, head and neck cancer, kidney chromophobe, kidney clear cell carcinoma, kidney papillary cell carcinoma, liver cancer, lung adenocarcinoma, lung squamous cell carcinoma, prostate cancer, rectal cancer, thyroid cancer, and uterine endometrial carcinoma, than in normal tissues (P<0.001) (*Figure 1*).

### *Prognostic significance of CD276 mRNA expression in various cancers*

As *CD276* mRNA expression was higher in cancer tissues than in normal tissues, we compared the OS between patients with various cancers exhibiting high and those exhibiting low *CD276* mRNA expression. We found that high *CD276* mRNA expression was associated with a poor prognosis in various cancers (*Table 1*). In particular, upregulated *CD276* mRNA expression was associated with

**Table 1** *CD276* mRNA expression is associated with poor OS in various cancers

| Cancer type | N | HR | 95% CI | P value |
|---|---|---|---|---|
| Adrenocortical carcinoma | 79 | 1.784 | 1.236–2.574 | 0.002 |
| Bladder cancer | 406 | 1.293 | 1.042–1.605 | 0.02 |
| Colon cancer | 455 | 1.406 | 1.033–1.914 | 0.03 |
| Glioblastoma multiforme | 151 | 1.603 | 1.172–2.193 | 0.003 |
| Head and neck cancer | 520 | 1.191 | 1.014–1.398 | 0.03 |
| Kidney chromophobe | 65 | 2.686 | 1.007–7.165 | 0.048 |
| Kidney clear cell carcinoma | 533 | 1.451 | 1.112–1.894 | 0.006 |
| Kidney papillary cell carcinoma | 289 | 1.937 | 1.069–3.510 | 0.03 |
| Lower-grade glioma | 514 | 2.250 | 1.782–2.840 | <0.001 |
| Liver cancer | 370 | 1.271 | 1.031–1.566 | 0.03 |
| Lung adenocarcinoma | 506 | 1.370 | 1.071–1.752 | 0.01 |
| Mesothelioma | 86 | 1.975 | 1.469–2.655 | <0.001 |
| Ocular melanoma | 80 | 2.052 | 1.152–3.653 | 0.02 |
| Pancreatic adenocarcinoma | 179 | 1.352 | 1.011–1.807 | 0.042 |

OS, overall survival; HR, hazard ratio; CI, confidence interval.

**Table 2** Patients' characteristics

| Characteristic | Value (N=86) |
|---|---|
| Age, years, mean | 63.1 |
| Sex, n (%) | |
| Male | 70 (81.4) |
| Female | 16 (18.6) |
| Stage, n (%) | |
| I | 10 (11.6) |
| II | 16 (18.6) |
| III | 44 (51.2) |
| IV | 16 (18.6) |
| Histology, n (%) | |
| Epithelioid | 57 (66.3) |
| Biphasic | 23 (26.7) |
| Sarcomatoid | 1 (1.2) |
| Unknown | 5 (5.8) |

a poor prognosis in adrenocortical carcinoma, bladder cancer, colon cancer, glioblastoma multiforme, head and neck cancer, kidney chromophobe, kidney clear cell carcinoma, kidney clear cell carcinoma, lower-grade glioma, liver cancer, lung adenocarcinoma, mesothelioma, ocular melanoma, and pancreatic adenocarcinoma (*Table 1*). These results indicate that *CD276* mRNA is a novel prognostic biomarker for these cancers.

### *Prognostic significance of CD276 mRNA expression in mesothelioma subtypes*

Owing to its association with a poor prognosis in various cancers, including mesothelioma, we investigated *CD276* mRNA expression as a prognostic biomarker in each mesothelioma subtype: epithelioid and nonepithelioid, which comprises biphasic and sarcomatoid mesothelioma. In particular, we assessed the association between clinical variables and *CD276* mRNA expression in 86 patients with mesothelioma. The characteristics of the patients (number of patients, age, sex, stage, histological evaluation, and high/low *CD276* mRNA expression) are described in *Table 2*. The OS analysis using the Kaplan-Meier method demonstrated

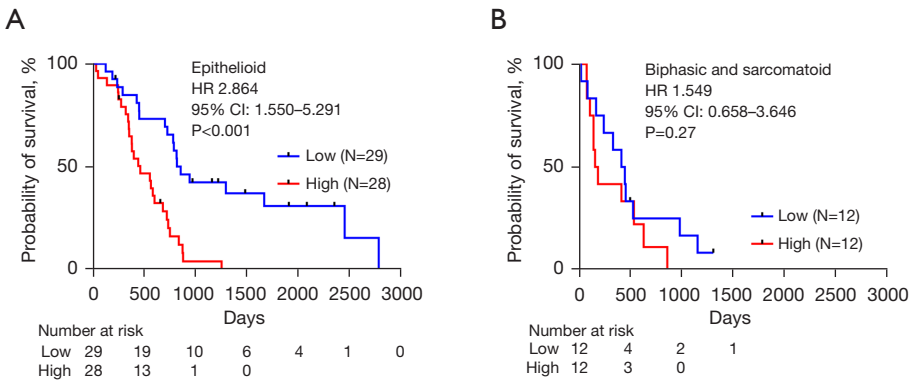


**Figure 2** *CD276* mRNA expression was associated with a poor prognosis in epithelioid mesothelioma. Overall survival analysis using the Kaplan-Meier method for *CD276* expression in epithelioid (A) and biphasic and sarcomatoid (B) mesothelioma. The log-rank test demonstrated that upregulated *CD276* mRNA expression was associated with a poor prognosis in epithelioid mesothelioma (HR =2.864, 95% CI: 1.550–5.291, P<0.001), whereas it was not associated with a poor prognosis in biphasic and sarcomatoid mesothelioma (HR =1.549, 95% CI: 0.658–3.646, P=0.27). HR, hazard ratio; CI, confidence interval.

that upregulated *CD276* mRNA expression was associated with a poor prognosis in epithelioid mesothelioma (HR =2.864, 95% CI: 1.550–5.291, P<0.001, *Figure 2A*), whereas it was not associated with a poor prognosis in biphasic and sarcomatoid mesothelioma (HR =1.549, 95% CI: 0.658–3.646, P=0.27, *Figure 2B*). As *CD276* mRNA expression was associated with a poor prognosis in epithelioid mesothelioma, we performed further analysis of OS using a multivariate Cox proportional hazards model. We found that upregulated *CD276* mRNA expression was associated with worse OS after adjusting the model for age, sex, and stage (HR =4.157, 95% CI: 2.014–8.921, *Table 3*), even when considering the currently available molecular-targeted therapeutic strategies. Therefore, our results suggest that *CD276* mRNA expression is an independent prognostic biomarker and therapeutic target in epithelioid mesothelioma.

### *Correlation between the mRNA expression of CD276 and expression of genes of each pathway in epithelioid mesothelioma*

Our study demonstrated that *CD276* mRNA expression is an independent prognostic biomarker and therapeutic target in epithelioid mesothelioma. Therefore, we explored the correlation between *CD276* and the gene markers of TIICs and each pathway involved in epithelioid mesothelioma (*Table 4*). *CD276* mRNA expression weakly correlated with the expression of 3 of the 37 markers of TIICs in epithelioid mesothelioma (*Table 4*). These results suggest that *CD276* mRNA expression does not correlate with various TIICs in epithelioid mesothelioma. However, we identified a strong positive correlation between *CD276* and *PKM2* expression (r=0.603, 95% CI: 0.399–0.749, P<0.001). We also identified moderate positive correlations between the expression of *CD276* and *SLC2A1* (r=0.423, 95% CI: 0.175–0.620, P=0.001), *HK1* (r=0.544, 95% CI: 0.323–0.709, P<0.001), *GAPDH* (r=0.440, 95% CI: 0.195–0.633, P=0.001), *SLC3A2* (r=0.420, 95% CI: 0.171–0.618, P=0.001), *SLC38A1* (r=0.489, 95% CI: 0.255–0.669, P<0.001), and *VEGFC* (r=0.473, 95% CI: 0.234–0.657, P<0.001) markers in epithelioid mesothelioma (*Table 5*, Table S1). These results illustrate that *CD276* mRNA expression is associated with the glycolytic pathway. In addition, we confirmed the factors influencing the correlation between the expression of *CD276* and that of *SLC2A1*, *HK1*, *GAPDH*, *PKM2*, *PDP1*, and *LDHB* in the glycolytic pathway using a multivariate linear regression model. After adjusting for six gene markers, we observed that the expression of *CD276* mostly correlated with that of *PKM2* (β=0.443, 95% CI: 0.087–0.799, P=0.01) (*Table 6*). *PKM2* plays an important role in generating two molecules of adenosine triphosphate (ATP) in the glycolytic pathway, which is essential for metabolic reprogramming during cancer progression. Our results show that *CD276* mRNA expression may regulate the glycolytic pathway in epithelioid mesothelioma.

**Table 3** Multivariate Cox proportion hazards model for OS demonstrating elevated *CD276* mRNA expression as an independent prognostic biomarker and therapeutic target in epithelioid mesothelioma

| Model | HR | 95% CI |
|---|---|---|
| Model 1 (*CD276*) | | |
| Target | 4.157 | 2.014–8.921 |
| Age | 1.026 | 0.988–1.066 |
| Sex | 0.856 | 0.391–1.757 |
| Stage | 0.83 | 0.565–1.237 |
| Model 2 (*PDCD1*) | | |
| Target | 0.843 | 0.456–1.553 |
| Age | 0.999 | 0.965–1.035 |
| Sex | 1.259 | 0.583–2.550 |
| Stage | 0.912 | 0.632–1.326 |
| Model 3 (*CTLA4*) | | |
| Target | 1.167 | 0.642–2.129 |
| Age | 0.999 | 0.965–1.035 |
| Sex | 1.259 | 0.582–2.560 |
| Stage | 0.886 | 0.617–1.295 |
| Model 4 (*VEGFA*) | | |
| Target | 1.58 | 0.829–3.035 |
| Age | 0.999 | 0.967–1.034 |
| Sex | 1.029 | 0.465–2.154 |
| Stage | 0.875 | 0.608–1.275 |
| Model 5 (*CD276* + *PDCD1*) | | |
| Target | 2.297 | 1.025–5.021 |
| Age | 1.008 | 0.972–1.046 |
| Sex | 0.871 | 0.372–1.909 |
| Stage | 0.886 | 0.617–1.289 |
| Model 6 (*CD276* + *CTLA4*) | | |
| Target | 2.36 | 1.108–4.872 |
| Age | 1.01 | 0.975–1.047 |
| Sex | 1.242 | 0.572–2.530 |
| Stage | 0.796 | 0.534–1.194 |
| Model 7 (*CD276* + *VEGFA*) | | |
| Target | 2.455 | 1.215–4.833 |
| Age | 1.013 | 0.978–1.050 |
| Sex | 1.021 | 0.469–2.085 |
| Stage | 0.912 | 0.622–1.360 |

OS, overall survival; HR, hazard ratio; CI, confidence interval.

## Discussion

### *Key findings*

In this study, upregulated *CD276* mRNA expression correlated with a poor prognosis, and it was confirmed as an independent prognostic biomarker and therapeutic target in epithelioid mesothelioma. In addition, we demonstrated that *CD276* mRNA expression may regulate the expression of genes associated with the glycolytic pathway, particularly *PKM2*.

Upregulated *CD276* mRNA expression was associated with a poor prognosis in patients with epithelioid mesothelioma (*Figure 2A*,*2B*). *CD276* mRNA expression was associated with a poor OS in epithelioid mesothelioma, as determined using the multivariate Cox proportional hazards model, even when available therapeutic strategies targeting *PDCD1*, *CTLA4*, and *VEGFA* were taken into consideration. Therefore, CD276 is an independent prognostic biomarker and a therapeutic target for epithelioid mesothelioma.

### *Strengths and limitations*

Our study highlighted the significance of *CD276* expression in epithelioid mesothelioma, which is a rare and refractory cancer. However, it had some limitations. First, as our study was retrospective and had a limited number of patients, prospective studies with larger populations will be necessary to validate the results. Second, our results were based on a clinical database; therefore, *in vivo* and *in vitro* studies will be necessary to clarify the significance and role of CD276 in epithelioid mesothelioma.

### *Comparison with similar research and explanations of findings*

High expression of *CD276* is correlated with a poor prognosis in mesothelioma, however, its subtypes and multivariate Cox proportional hazards models were not considered in these analyses (31,32). Further, age, sex, and stage were not associated with the prognosis of epithelioid mesothelioma, as determined using the multivariate Cox proportional hazards model. However, it should be noted that in multivariate analysis, a strong prognostic factor may mask the effects of other potential prognostic variables (35). As *CD276* mRNA expression was identified to be a strong factor influencing OS, age, sex, and stage may not be prognostic markers.

**Table 4** Correlation between *CD276* mRNA expression and that of gene markers of TIICs in epithelioid mesothelioma

| Immune cells | Gene markers | r | 95 % CI | P value |
|---|---|---|---|---|
| Immune checkpoint | *CD274* | –0.001 | –0.268 to 0.267 | 0.99 |
| | *PDCD1* | 0.194 | –0.078 to 0.439 | 0.14 |
| | *CTLA4* | 0.147 | –0.126 to 0.399 | 0.27 |
| | *ICOS* | 0.037 | –0.233 to 0.302 | 0.78 |
| Macrophages | *CD68* | 0.041 | –0.229 to 0.306 | 0.76 |
| M1-type (classically activated) macrophages | *NOS2* | 0.282 | 0.0150 to 0.511 | 0.03* |
| M2-type (alternatively activated) macrophages | *ARG1* | –0.004 | –0.271 to 0.264 | 0.97 |
| | *MRC1* | 0.159 | –0.113 to 0.410 | 0.23 |
| Tumor-associated macrophages | *HLA-G* | –0.047 | –0.311 to 0.223 | 0.72 |
| | *CD80* | 0.142 | –0.131 to 0.395 | 0.29 |
| | *CD86* | 0.052 | –0.219 to 0.315 | 0.70 |
| Monocytes | *CD14* | –0.118 | –0.374 to 0.155 | 0.38 |
| Natural killer cells | *XCL1* | –0.163 | –0.412 to 0.110 | 0.22 |
| | *KIR3DL1* | –0.257 | –0.491 to 0.012 | 0.054 |
| | *CD7* | –0.023 | –0.288 to 0.247 | 0.86 |
| Neutrophils | *MPO* | 0.014 | –0.254 to 0.281 | 0.91 |
| Dendritic cells | *CD1C* | –0.100 | –0.358 to 0.173 | 0.46 |
| B-cells | *CD19* | –0.289 | –0.516 to –0.022 | 0.03* |
| | *CD38* | –0.159 | –0.409 to 0.114 | 0.23 |
| $CD8^+$ T-cells | *CD8A* | 0.079 | –0.193 to 0.340 | 0.55 |
| | *CD8B* | 0.149 | –0.123 to 0.401 | 0.26 |
| Follicular helper T-cells | *CXCR5* | –0.026 | –0.291 to 0.244 | 0.85 |
| | *BCL6* | 0.117 | –0.155 to 0.373 | 0.38 |
| T helper-1 cells | *IL12RB2* | 0.127 | –0.145 to 0.382 | 0.34 |
| T helper-2 cells | *CCR3* | 0.053 | –0.217 to 0.317 | 0.69 |
| | *STAT6* | –0.387 | –0.593 to –0.133 | 0.003** |
| | *GATA3* | 0.030 | –0.239 to 0.296 | 0.82 |
| T helper-9 cells | *TGFBR2* | 0.007 | –0.261 to 0.275 | 0.95 |
| | *IRF4* | –0.169 | –0.418 to 0.103 | 0.20 |
| | *SPI1* | –0.019 | –0.285 to 0.250 | 0.88 |
| T helper-17 cells | *IL-21R* | 0.129 | –0.144 to 0.384 | 0.33 |
| | *IL-23R* | –0.163 | –0.412 to 0.110 | 0.22 |
| T helper-22 cells | *CCR10* | 0.186 | –0.086 to 0.432 | 0.16 |
| | *AHR* | –0.035 | –0.300 to 0.235 | 0.79 |
| Regulatory T-cells | *FOXP3* | 0.120 | –0.152 to 0.376 | 0.37 |
| | *CCR8* | 0.161 | –0.111 to 0.411 | 0.23 |

**, P<0.01; *, P<0.05. TIICs, tumor-infiltrating immune cells; CI, confidence interval.

**Table 5** Correlation between mRNA expression of *CD276* and expression of gene markers of the glycolytic pathway in epithelioid mesothelioma

| Pathway | Gene marker | r | 95% CI | P value |
|---|---|---|---|---|
| Glycolytic | *SLC2A1* | 0.423 | 0.175 to 0.620 | 0.001** |
| | *HK1* | 0.544 | 0.323 to 0.709 | <0.001*** |
| | *GPI* | 0.152 | –0.121 to 0.403 | 0.26 |
| | *PFKP* | 0.199 | –0.072 to 0.444 | 0.13 |
| | *PFKL* | 0.241 | –0.028 to 0478 | 0.070 |
| | *ALDOA* | –0.060 | –0.323 to 0.211 | 0.65 |
| | *GAPDH* | 0.440 | 0.195 to 0.633 | 0.001** |
| | *PGK1* | 0.164 | –0.109 to 0.414 | 0.22 |
| | *PGAM5* | 0.263 | –0.005 to 0.496 | 0.048* |
| | *BPGM* | –0.101 | –0.359 to 0.171 | 0.45 |
| | *ENO1* | 0.136 | –0.137 to 0.389 | 0.31 |
| | *ENO2* | 0.332 | 0.070 to 0.551 | 0.01* |
| | *ENO3* | 0.055 | –0.216 to 0.318 | 0.68 |
| | *PKM2* | 0.603 | 0.399 to 0.749 | <0.001*** |
| | *PDP1* | 0.299 | 0.034 to 0.525 | 0.02* |
| | *LDHA* | 0.001 | –0.267 to 0.269 | 0.99 |
| | *LDHB* | 0.336 | 0.075 to 0.554 | 0.01* |
| | *ME2* | 0.123 | –0.150 to 0.378 | 0.36 |
| | *PCK1* | –0.172 | –0.421 to 0.100 | 0.20 |

***, P<0.001; **, P<0.01; *, P<0.05. CI, confidence interval.

**Table 6** Correlation between mRNA expression of *CD276* and expression of gene markers of the glycolytic pathway in epithelioid mesothelioma using a multivariate linear regression model

| Gene markers | β | 95% CI | P value |
|---|---|---|---|
| *SLC2A1* | 0.088 | –0.039 to 0.216 | 0.17 |
| *HK1* | 0.182 | –0.044 to 0.408 | 0.11 |
| *GAPDH* | –0.103 | –0.446 to 0.240 | 0.54 |
| *PKM2* | 0.443 | 0.087 to 0.799 | 0.01 |
| *PDP1* | 0.087 | –0.131 to 0.305 | 0.42 |
| *LDHB* | 0.144 | –0.112 to 0.401 | 0.26 |

β, regression coefficient; CI, confidence interval.

*CD276* mRNA expression does not correlate with various TIICs in epithelioid mesothelioma including *PDCD1* and *CTLA4* mRNA, which are the existing therapeutic target for mesothelioma, except for the expression of 3 of the 37 markers of TIICs. Further, the correlation of 3 markers of TIICs were weak correlation in epithelioid mesothelioma. In addition, *CD276* mRNA expression does not correlate with *VEGFA* mRNA, which is another existing therapeutic target for mesothelioma. In fact, *CD276* mRNA expression was independent prognostic biomarker using the multivariate Cox proportional hazards model, considering *PDCD1*, *CTLA4*, and *VEGFA* in epithelioid mesothelioma.

Based on these results, *CD276* mRNA may be the independent immune checkpoint different from immune checkpoint with existing ICIs and therapeutic target in epithelioid mesothelioma.

Notably, *CD276* mRNA expression correlated well with gene markers of the glycolytic pathway (*Table 5*). This result indicates that CD276 regulates the glycolytic pathway in epithelioid mesotheliomas. The strongest correlation was identified between *CD276* and *PKM2*, and a moderate correlation was identified between *CD276* and *SLC2A1* and *HK1* (*Table 5*). *PKM2* generates two ATP molecules via this pathway, which is essential for metabolic reprogramming for cancer progression (36). Thus, *CD276* mRNA expression is strongly associated with *PKM2* expression and ATP generation in the glycolytic pathway of epithelioid mesotheliomas. In addition, PKM2 and HK1, which encode enzymes that control two of the three irreversible reactions in the glycolytic pathway, play crucial roles (36) after glucose intake into the cytoplasm via GLUT1, encoded by *SLC2A1* (37). However, irreversible reactions, including those controlled by *PDP1*, which weakly correlated with *CD276* mRNA expression, lead to the tricarboxylic acid (TCA) cycle for ATP generation for metabolic reprogramming (38). A recent study has demonstrated that cancer cells rely on the TCA cycle and the glycolytic pathway for ATP generation (38). Therefore, our results suggest that *CD276* mRNA expression may affect the TCA cycle and glycolytic pathway. Increased levels of PKM1, an enzyme that converts phosphoenolpyruvate to pyruvate, similar to PKM2, lead to upregulation of the glycolytic pathway and induction of the TCA cycle (39). However, the dataset used in this study did not include data on *PKM1* mRNA expression, warranting studies on the correlation between *CD276* and *PKM1* in epithelioid mesotheliomas.

### *Implications and actions needed*

Although evidence suggests that *CD276* is associated with ferroptosis (24); angiogenesis (25); and the JAK2/STAT3, PIK3CA/AKT/mTOR, and RAF/MEK pathways (26-28) in various cancer, our results illustrated that *CD276* expression was not well associated with these pathways in epithelioid mesothelioma. Based on the results of a previous *in vivo* study (33) and our study, *CD276* is a target for ICIs in epithelioid mesothelioma and contributes to achieving better OS in patients with epithelioid mesothelioma.

## Conclusions

Our study indicated that *CD276* mRNA expression is associated with poor prognosis in various cancers, especially epithelioid mesothelioma. Moreover, *CD276* mRNA expression was identified to be an independent prognostic biomarker in epithelioid mesothelioma. Our results showed that *CD276* mRNA expression correlated with gene markers of the glycolytic pathway, especially *PKM2*. Thus, *CD276* could be associated with the glycolytic pathway and contribute to ATP generation in epithelioid mesotheliomas. Based on these findings, CD276 is a target for ICIs in future therapeutic approaches; CD276 inhibitors could improve prognosis in patients with epithelioid mesothelioma. An ICIs against CD276 should be confirmed for obtaining a better prognosis in patients with epithelioid mesothelioma in the near future.

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## Footnote

*Reporting Checklist:* The authors have completed the REMARK reporting checklist. Available at https://jtd.amegroups.com/article/view/10.21037/jtd-24-1598/rc

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*Ethical Statement:* The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was

conducted in accordance with the Declaration of Helsinki (as revised in 2013). Ethical approval for the study was not required as we used data from an anonymized public open database and individual consent for this retrospective analysis was waived by the Institutional Review Board of Tokyo Women's Medical University.



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