LAMC2 as a prognostic biomarker in human cancer: a systematic review and meta-analysis

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INTRODUCTION

Laminins are trimeric proteins composed of α, β and γ chains, which are the main component of basement membranes.1 Mammalian genome encodes five α chains, four β chains and three γ chains.2 Loss-of-function studies show that most laminin mutants are embryonic lethal.3 Laminins are involved in various biological processes, including cellular phenotype maintenance, adhesion, migration, growth and differentiation in vivo and in vitro.4,5

In recent years, laminin γ2 (LAMC2) has attracted increasingly attractive because of the aberrant expression of LAMC2 in various cancer. The LAMC2 expression was significantly upregulated in colorectal cancer tissues compared with adjacent normal tissues, and high LAMC2 expression is known to be associated with poor patient prognosis.6 Furthermore, overexpression of LAMC2 has been reported in pancreatic ductal adenocarcinoma,7,11 non-small cell lung cancer,12 penile squamous cell carcinoma,13 ovarian cancer,14 oral tongue squamous cell carcinoma,15 cholangiocarcinoma16 and oesophageal squamous cell carcinoma,17 leading to poor clinicopathological features and short survival time.

However, individual studies may be inadequate and inaccurate due to their small sample and study design. To date, there was no meta-analysis has been performed to investigate the relationship between LAMC2 and the prognostic value. Therefore, we performed a comprehensive meta-analysis to assess the correlation between LAMC2 and survival outcomes and clinicopathological features in human cancers.
METHODS
This study followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses guidelines \(^{18}\) (online supplemental table S1).

Patient and public involvement
No patient was involved.

Search strategy
A literature search was conducted in Embase, Web of Science and PubMed (up to 25 November 2021). The keywords were (laminin C2 OR LAMC2 OR laminin subunit gamma 2) AND (prognosis OR prognostic OR survival). The detailed search strategy is in online supplemental file 1. The reference lists and citation sections of relevant studies were also screened for additional eligible studies.

Study selection
Studies that met the following criteria were included in the meta-analysis: (1) study of the relationship between LAMC2 and prognosis of cancers, (2) patients were divided into two groups: high LAMC2 expression and low LAMC2 expression group, and (3) associations of LAMC2 expression with overall survival (OS) and clinicopathological features were described. The exclusion criteria in our meta-analysis were as follows: (1) case reports, letters, reviews, editorials and expert opinions, (2) studies published non-English language, (3) non-human studies and (4) studies without sufficient available data.

Quality assessment
The Newcastle-Ottawa Scale (NOS) criteria were used to assess the quality of the eligible studies.\(^ {19}\) The NOS contains nine items that include selection, comparability and outcome for studies. When the NOS score was ≥6, the study was considered high quality.

Data extraction
Two authors (TF and ZY) performed the data extraction independently. Disagreements were resolved by discussion and consensus with the third author (JX). The following information from the included studies was collected: the first author’s name, publication year, country, number of cases, cancer type, the detection method of LAMC2, clinicopathological features and survival outcome. When multivariate and univariate analyses were simultaneously reported, only the former was extracted. If studies only provided Kaplan-Meier curves, the survival data were extracted from the graphical curve and calculated HR and 95% CI were reckoned using the published method.\(^ {20}\)

Public data and tools
The web-based tool named Gene Expression Profiling Interactive Analysis (GEPIA) was used to analyse associations between LAMC2 and clinical outcomes.\(^ {21}\)

Statistical analysis
We used Stata MP V.16 software (Stata) to perform statistical analysis. Log OR and 95% CI were calculated for the association of LAMC2 expression and clinicopathological characteristics. The prognostic role of LAMC2 expression in OS, disease-specific survival (DSS) and progression-free survival (PFS) was evaluated through HR with 95% CI. The statistical heterogeneity among the studies was analysed by using I² test and Q test. When significant heterogeneity (I²≥50%, p < 0.05) was observed, the random-effects model was chosen. Otherwise, the fixed-effects model was used. The Egger's test and Begg's test were used to assess the potential publication bias. We conducted a sensitivity analysis to explore the stability of the overall meta-analysis results. p<0.05 was considered as statistically significant.

RESULTS
Study identification and characteristics
As is shown in figure 1, a total of 7 studies with 1056 patients with cancer were included in this meta-analysis satisfied the inclusion criteria.\(^ {6 7 13 16 17 22 23}\) The publication period ranged from 2017 to 2021. All of our included studies had high quality with NOS ≥6. The included studies addressed six different cancer types: oesophageal squamous cell carcinoma (n=1), pancreatic ductal adenocarcinoma (n=2), cholangiocarcinoma (n=1), papillary thyroid cancer (n=1), colorectal cancer (n=1) and penile squamous cell carcinoma (n=1). The main characteristics of the seven studies are summarised in table 1.

Relationship between LAMC2 expression and clinicopathological parameters
We evaluated the relationship between LAMC2 expression and clinicopathological parameters in...
various cancers. As shown in figure 2A,B, there were three and six studies describing patients of age and gender, respectively. The pooled analysis demonstrated that there was no significant association between LAMC2 expression and age or gender. High expression of LAMC2 was significantly associated with lymph node metastasis (LNM) (log OR 0.88, 95% CI 0.38 to 1.38, p=0.001, figure 2C) and tumour-node-metastasis (TNM) stage (log OR: 0.95, 95% CI 0.39 to 1.50, p=0.001, figure 2D). There was significant heterogeneity among these studies ($I^2=59.61\%$, $p=0.02$; $I^2=60.16\%$, $p=0.03$), and thus the random-effects DerSimonian-Laird model was adopted. A total of four studies evaluated tumour status according to LAMC2 expression. No statistically significant heterogeneity was found among the studies ($I^2=36.00\%$, $p=0.20$); thus, the fixed-effect Mantel-Haenszel model was adopted. As shown in figure 2E, high expression of LAMC2 was significantly associated with tumour status (log OR 1.26, 95% CI 0.84 to 1.68, p=0.001).

### Table 1 Characteristics of studies included in this meta-analysis

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>Region</th>
<th>Sample</th>
<th>Cancer</th>
<th>Method</th>
<th>Outcome</th>
<th>NOS score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Okada et al.</td>
<td>2021</td>
<td>Japan</td>
<td>114</td>
<td>Pancreatic ductal adenocarcinoma</td>
<td>qRT-PCR</td>
<td>OS</td>
<td>8</td>
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<tr>
<td>Okada et al.</td>
<td>2021</td>
<td>Japan</td>
<td>121</td>
<td>Pancreatic ductal adenocarcinoma</td>
<td>qRT-PCR</td>
<td>OS</td>
<td>8</td>
</tr>
<tr>
<td>Pei et al.</td>
<td>2019</td>
<td>China</td>
<td>121</td>
<td>Cholangiocarcinoma</td>
<td>IHC</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>Liang et al.</td>
<td>2018</td>
<td>China</td>
<td>64</td>
<td>Oesophageal squamous cell carcinoma</td>
<td>qRT-PCR</td>
<td>OS</td>
<td>7</td>
</tr>
<tr>
<td>Zhan et al.</td>
<td>2019</td>
<td>China</td>
<td>473</td>
<td>Papillary thyroid cancer</td>
<td>RNA-seq</td>
<td>PFS</td>
<td>6</td>
</tr>
<tr>
<td>Zhou et al.</td>
<td>2018</td>
<td>China</td>
<td>114</td>
<td>Penile squamous cell carcinoma</td>
<td>IHC</td>
<td>DSS</td>
<td>7</td>
</tr>
<tr>
<td>Huang et al.</td>
<td>2017</td>
<td>China</td>
<td>49</td>
<td>Colorectal cancer</td>
<td>IHC</td>
<td></td>
<td>6</td>
</tr>
</tbody>
</table>

DSS, disease-specific survival; IHC, immunocytochemistry; NOS, Newcastle-Ottawa scale; OS, overall survival; PFS, progression-free survival; qRT-PCR, quantitative real-time PCR.

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**Figure 2** Forest plots of studies evaluating the relationship between LAMC2 expression and clinicopathological features. (A) LNM, (B) TNM stage, (C) Tumour status, (D) Gender, (E) Age. LNM, lymph node metastasis; TNM, tumour-node-metastasis.
This finding suggests that high LAMC2 expression is associated with clinicopathological features.

**Relationship between LAMC2 expression and OS/PFS/DSS**

There were 5 studies including 872 patients presenting the relationship between LAMC2 and OS/PFS/DSS. Due to no significant heterogeneity being found among studies ($I^2=0.00\%$, $p=0.83$), the fixed-effect inverse-variance model was adopted to estimate the pooled HR and 95% CI. The pooled HR indicated that the expression of LAMC2 was negatively associated with OS/PFS/DSS (HR 1.85, 95% CI 1.31 to 2.40, $p<0.001$, figure 3A), which demonstrated that LAMC2 was a risk factor for the prognosis of patients with cancer. In addition, sensitivity analysis was performed to determine the effect of individual studies on the OS/PFS/DSS. It revealed that no single study altered the pooled LAMC2 HR result significantly (figure 3B). This suggested that the result of the meta-analysis was stable.

**Analysis of publication bias**

Funnel plot (figure 3C), Beggs’s test and Egger’s linear regression test (figure 3D) were used to assess publication bias. The results showed that the funnel plots scatter symmetrically. The statistical tests showed $p$ values were greater than 0.05 (Beggs’s test: $p=0.4624$; Egger’s test: $p=0.329$). Thus, there was no obvious publication bias in the prognostic meta-analysis.

**Validation of TCGA data set results**

To validate our result, we retrieved LAMC2 expression data and clinical data from the TCGA dataset. As shown in figure 4A, LAMC2 was increased in colon adenocarcinoma (COAD), lymphoid neoplasm diffuse large B-cell lymphoma, oesophageal carcinoma, head and neck squamous cell carcinoma (HNSC), lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC), determined using a log2FC cut-off of 1 and a $p$ value cut-off of 0.01. A total of 9502 patients with urinary, digestive, female reproductive, respiratory and blood systems cancers were included in the survival analysis from the TCGA database. According to the expression of LAMC2, the patients were divided into high and low groups using the median score as the cut-off by GEPIA.21 The results showed that higher LAMC2 expression was correlated with worse survival (figure 4B). We also explored the prognostic role of LAMC2 in different cancer types. As shown in figure 5, LAMC2 expression was significantly associated with OS in LUAD (figure 5A), mesothelioma (MESO, figure 5B), skin cutaneous melanoma (SKCM, figure 5C), HNSC (figure 5D) and brain lower grade
glioma (LGG, figure 5E). However, LAMC2 expression was not related to OS in LUSC (figure 5F).

**DISCUSSION**

Laminins are involved in various cancer development and prognosis.24,25 Increasing evidence suggests that the various laminin isoforms could be useful biomarkers of cancer diagnosis and might be potential therapeutic targets for cancers treatment, such as LAMA5,14 LAMB1,26 and LAMC1.27 LAMC2 is encoding by Laminin γ2 and has been confirmed as a therapeutic target for cancers. Here we performed a meta-analysis of seven studies to achieve a comprehensive evaluation between higher LAMC2 expression and clinicopathological characteristics in various cancer types. Our results showed that higher LAMC2 expression was significantly associated with LNM, TNM stage and tumour status. However, there was no significant association between LAMC2 expression and age or gender. Simultaneously, we also found that OS/PFS/DSS was higher in patients with low LAMC2 expression. Publication bias analysis demonstrated that no publication bias was observed among the included studies. These results suggested that LAMC2 might be a valuable biomarker for predicting prognosis in patients with cancer. Steyerberg et al provide the prognostic model that can provide effective solving discrimination and predictiveness measures.28 We will plan to investigate this in a later study.

Several kinds of researches have shown that LAMC2 promotes cancer cells proliferation, motility and invasion.29-33 However, the specific mechanisms are not particularly well understood. There is a study indicating that ZNF750 inhibited the migration of oesophageal
squamous cancer cells by inhibiting the LAMC2 transactivation. In hepatocellular carcinoma (HCC), LAMC2 has been found to be regulated by miR-548c-3p and inhibited the epithelial-mesenchymal transition in HCC. In pancreatic cancer cells, LAMC2 promoted Akt-Ser473 phosphorylation and increased expression and cell membrane accumulation of NHE1, promoting cell migration and invasion. A study by Wu et al showed that the LAMP3-LAMC2-TNC signal regulated the efficacy of radiation exposure in laryngeal squamous cell carcinoma. High-throughput sequencing results showed that miR-338-5p/3p targets LAMC2 to suppress invasion in salivary adenoid cystic carcinoma cells. LAMC2 was found to promote tumour progression by EGFR signalling. The latest research showed that overexpression of LAMC2 enhanced pancreatic ductal adenocarcinoma metastasis and tumourigenesis through the EGFR/ERK1/2/AKT/mTOR signalling pathway. These findings suggested that LAMC2 might play as an oncogene and predict prognosis in patients with cancer.

Recent researches have also investigated LAMC2 as a valuable biomarker of cancer diagnosis. Due to a limitation of the small sample size, we explored the expression of LAMC2 in various cancer types using (The Cancer Genome Atlas) TCGA database. The results showed that LAMC2 was upregulated in tumours and might be used as a biomarker for a variety of tumour types. Moreover, we explored the survival analysis from the TCGA database. The results demonstrated that higher LAMC2 expression was associated with poor OS in 9502 patients. We also explored the prognostic role of LAMC2 in different types of cancer. LAMC2 expression was significantly associated with OS in LUAD, MESO, SKCM, HNSC and LGG, but not in LUSC. This deserves further investigation.

Some potential limitations of our study should be noted. First, only seven articles were included in our meta-analysis, the limited number of studies might influence the reliability of the results. Of the seven included studies, five were from China and two were from Japan. So, our results may only be applicable to the Asian population. Although we determined data from the TCGA database, future studies from non-Asian populations are needed to confirm our findings. Second, there was no consensus on a cut-off value for higher LAMC2 expression. Third, in some researches, the data for HR and 95% CI value was not provided. Although we tried our best to extract the HR and 95% CI value from the Kaplan-Meier curve, some errors are inevitable. Fourth, the numbers of patients and tumour types included in this meta-analysis were still limited. So, our results may exaggerate the prognostic value of LAMC2.

**CONCLUSIONS**

In conclusion, LAMC2 may be a valuable biomarker for cancer diagnosis, and upregulation of LAMC2 is
associated with a poor prognosis in patients with cancer. And increased LAMC2 expression is significantly associated with bad tumour status, TNM stages and LNM. For future clinical applications, more high-quality studies with large sample sizes are needed to confirm the role of LAMC2 in various cancers and regions.

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Contributors TF and ZY designed the study; TF, ZY and JX searched the literature and extracted data; ZY prepared the figures; ZY, J-XL and ZG wrote the manuscript. All authors gave the final approval for the paper to be published. ZY and TF act as guarantors.

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Ethics approval Not applicable.

Provenance and peer review Not commissioned; externally peer reviewed.

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Search strategy for PubMed (search date: from inception to 25 November 2021).


Search strategy for the Embase (search date: from inception to 25 November 2021).

'"laminin c2' OR (("laminin'/exp OR laminin) AND ("c2'/exp OR c2)) OR lamic2 OR 'laminin subunit gamma 2'/exp OR 'laminin subunit gamma 2' OR (("laminin'/exp OR laminin) AND subunit AND gamma AND ("2'/exp OR 2))' AND ("prognosis'/exp OR prognosis OR prognostic OR 'survival'/exp OR survival)"

Search strategy for Web of Science (search date: from inception to 25 November 2021)

TS=(("laminin C2 OR LAMC2 OR laminin subunit gamma 2) AND (prognosis OR prognostic OR survival))