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**Original Article**

# Claudin 18.2 Expression in Colorectal Mucinous Adenocarcinoma: A Clinicopathological Analysis

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**Abstract:**

**Background:** Colorectal mucinous adenocarcinoma (MAC) is a distinct subtype of colorectal adenocarcinoma characterised by high recurrence rate and limited treatment options. The tight-junction-associated protein claudin 18.2 (CLDN18.2) has emerged as a novel therapeutic target in various solid tumours. This study aimed to assess CLDN18.2 expression in MAC and its clinicopathological association.

**Methods:** CLDN18.2 expression was immunohistochemically evaluated in a cohort of 91 patients with MAC who underwent surgical procedures between January 2022 and April 2024. Positive CLDN18.2 staining was defined as ≥10% of tumour cells exhibiting ≥1+ membrane staining or any instance of ≥2+ membrane staining.

**Results:** CLDN18.2 expression was detected in 19.8% (18/91) of the patients with MAC. Positive expression of CLDN18.2 significantly correlated with perineural invasion ($P$ = 0.0235) and lymphovascular invasion ($P$ = 0.0375). CLDN18.2 demonstrated frequent heterogeneous expression in MAC.

**Conclusions:** In summary, the present study found that CLDN18.2 was positively expressed in MAC, and its overexpression was associated with increased invasive capacity. Therefore, CLDN18.2 is a promising therapeutic target that warrants further investigation in patients with MAC.

**Keywords: Colorectal mucinous adenocarcinoma; claudin 18.2; expression; immunohistochemistry**

## 1. Introduction

Colorectal mucinous adenocarcinoma (MAC) is a distinct subtype of colorectal adenocarcinoma (CRA), characterised by pathological feature in which more than 50% of the tumour tissue consists of extracellular mucin. When the proportion of extracellular mucin is less than 50%, the tumour is classified as adenocarcinoma with mucinous features[1]. More than 90% of colorectal cancers (CRC) are classified as CRA, with MAC accounting for approximately 1.6%–25.4% of cases, according to estimates[2]. MAC exhibits clinical and pathological characteristics that differ substantially from those of non-mucinous adenocarcinoma (NMAC)[3]. Typically, MAC tumours have larger diameters and are diagnosed at more advanced stages according to the TNM classification. Additionally, MAC is less responsive to both radiotherapy and chemotherapy, resulting in a heightened risk of recurrence and poor overall prognosis[4]. Therefore, identifying new therapeutic targets is urgently needed.

Claudin 18, a pivotal member of the claudin family, is a crucial transmembrane component of tight junctions. Claudin 18 subtype 2 (CLDN18.2) is produced through alternative splicing of the

CLDN18 gene[5]. This protein is aberrantly activated during malignant transformation and is expressed in gastric, pancreatic, oesophageal, colorectal, and lung adenocarcinomas[6,7]. Its restricted expression in normal tissues, coupled with tumour-specific expression, renders CLDN18.2 an ideal target for the treatment of solid tumours. Several therapeutic agents targeting claudins have been developed, including zolbetuximab (Claudiximab, IMAB362), a monoclonal antibody that specifically targets claudin 18.2 and has demonstrated substantial therapeutic efficacy in gastric carcinoma[8,9].

In the current study, we assessed the expression of CLDN18.2 in MAC and analysed the relevant clinicopathological factors associated with this expression. The primary objective of this study was to investigate CLDN18.2 expression in MAC and explore new therapeutic possibilities for patients diagnosed with this condition.

## Materials and Methods

### Patients Collection

In this study, we included a cohort of 91 patients diagnosed with MAC who underwent surgical procedures at Chongqing University Three Gorges Hospital between January 2022 and August 2024. The histological diagnosis of MAC was based on the World Health Organization (5th edition) classification of colorectal tumours and all slides were confirmed by two senior pathologists. Patients were excluded from the cohort if they met any of the following criteria: (1) a mixed invasive NMAC component exceeding 50% or (2) insufficient tissue in surgical samples for further immunohistochemical (IHC) staining.

Clinical and pathological variables, including age, sex, tumour site, and histological grade, were systematically recorded. All haematoxylin and eosin and IHC-stained sections were reviewed by two independent pathologists. Various parameters were re-evaluated, such as cell morphology, histological grade, and IHC staining for the Ki67 index, MLH1, MSH2, MSH6, and PMS2. This study was approved by the Ethics Committee of the Chongqing University Three Gorges Hospital.

### *IHC Staining*

IHC staining was conducted using the DAKO Autostainer Link 48 (Agilent, Santa Clara, CA, USA), according to the manufacturer's protocol. Following deparaffinisation and rehydration, 4 µm thick formalin-fixed paraffin-embedded sections were subjected to antigen retrieval using ethylenediaminetetraacetic acid at 97 °C for 40 min, followed by cooling to room temperature. The sections were sequentially incubated at room temperature with a peroxidase blocker (K8002, Dako) for 5 min, followed by incubation with a primary antibody against CLDN18.2 (Ab222512, 1:500, Abcam, China) for 40 min and a secondary antibody (GB23303, 1:200, Servicebio, Dako) for 30 min. The sections were treated with diaminobenzidine tetrahydrochloride for 10 min and counterstained with haematoxylin for 3 s. Between each step, the sections were washed twice with phosphate-buffered saline (K8007, Dako). After staining, the sections were mounted and scanned electronically for subsequent analyses[10].

### *IHC Analysis*

The CLDN18.2 IHC score for each section was determined by assessing both the intensity of membrane staining and the proportion of positive tumour cells. Membrane staining was considered positive if it was present and without extending to the cytoplasm or nucleus. Staining intensity was categorised as negative (0), faint (1+), moderate (2+), or strong (3+). Nearby colorectal mucosal epithelial tissues were used as positive controls. Samples exhibiting ≥10% of tumour cells exhibiting a membrane staining intensity of ≥1+ or any instance of ≥2+ membrane staining were classified as CLDN18.2 positive. The IHC staining scores for MLH1, MSH2, MSH6, and PMS2 were assessed based on both intensity and proportion. All slides were evaluated independently by two trained pathologists, and any discrepancies were resolved through collaborative discussion.

### *Assessment of Heterogeneous Expression*

During the review of both IHC and H-scores, we observed substantial intratumoural heterogeneity in the MAC. Due to the absence of accredited guidelines for assessing heterogeneity among patients with MAC, we consulted relevant literature to inform our classification. We categorised heterogeneity based on IHC scores; specifically, if both 3+ and 0 scores were present in a single tumour tissue and accounted for more than 50% of the combined area, we deemed this to

indicate strong heterogeneous expression. Furthermore, we evaluated the immunostaining patterns of these heterogeneous tumours. Some tumour cells within the MAC displayed a diffuse distribution with low or absent IHC staining, which we described as "scattered".

### *Statistical Analysis*

All counting data are presented as numbers and percentages. Chi-square tests were used for the univariate analysis of clinicopathological variables. All statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY, USA), and *P*-values < 0.05 were deemed statistically significant. This work was supported by the Institutional Review Board of Chongqing University Three Gorges Hospital and all the research was performed in accordance with relevant guidelines.

## Results

### *Clinicopathological Features of Patients with MAC*

The basic clinical and pathological data of the 91 patients with CRC are presented in Table 1. The median age of the cohort was 64.8 years, it comprised 48 females (53%) and 43 males (47%). The most frequently observed tumour site was the rectum, accounting for 37% of cases, while tumours in the left colon accounted for 58%. Among the patients, 42 (46.2%) were classified as early-stage (stages I–II), while 49 (53.8%) were classified as late-stage (stage III–IV).

**Table 1. Patient characteristics and claudin 18.2 expression in colorectal mucinous adenocarcinoma.**

| **Clinicopathological characteristics** | **Total (N = 91)** | **Claudin 18.2 expression** | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | ***P*-value** |
| **Age (years), N (%)** | | 18 | 73 | 0.12 |
| ≤65 | 41 (45.1) | 5 (27.8) | 36 (49.3) | |
| >65 | 50 (54.9) | 13 (72.2) | 37 (50.7) | |
| **Sex, N (%)** | | | | 0.6073 |
| Female | 48 (52.7) | 8 (44.4) | 38 (52.1) | |
| Male | 43 (47.3) | 10 (55.6) | 35 (47.9) | |
| **Tumour sites, N (%)** | | | | 0.19 |
| Right colon | 33 (36.3) | 9 (50.0) | 24 (32.9) | |
| Left colon | 58 (63.4) | 9 (50.0) | 49 (67.1) | |
| Rectum | 37 (40.6) | 6 (33.3) | 31 (42.5) | |
| **pT stage, N (%)** | | | | >0.99 |
| pT1-T2 | 13 (14.3) | 2 (11.1) | 11 (15.1) | |
| pT3-T4 | 78 (85.7) | 16 (88.9) | 62 (85.0) | |
| **Lymph node metastasis, N (%)** | | | | 0.79 |
| Yes | 42 (46.2) | 9 (50) | 33 (45.2) | |
| No | 49 (53.8) | 9 (50) | 40 (54.8) | |
| **Distant metastasis, N (%)** | | | | 0.76 |
| Yes | 21 (23.1) | 5 (27.8) | 16 (21.9) | |
| No | 70 (76.9) | 13 (72.2) | 57 (78.1) | |
| **AJCC TNM stage, N (%)** | | | | 0.60 |
| I–II | 42 (46.2) | 7 (38.9) | 35 (47.9) | |
| III–IV | 49 (53.8) | 11 (61.1) | 38 (52.1) | |
| **Perineural invasion, N (%)** | | | | **0.024** |
| Yes | 10 (11.0) | 5 | 5 | |
| No | 81 (89.0) | 13 | 68 | |

***Expression of CLDN18.2***

Among patients with MAC, 18 exhibited positive staining for CLDN18.2, resulting in a positivity rate of 19.8%. Low expression of CLDN18.2 was defined as a membrane staining intensity score in less than 40% of tumour cells with a value of ≥2+. Moderate expression was defined as a score between 40% and 70% of tumour cells with a value of ≥2+, while high expression was defined as a score exceeding 70% of tumour cells with a value of ≥2+. Representative images are shown in Figure 1. Among patients with CLDN18.2-positive, one (5%) exhibited high expression, four (21%) exhibited low expression, and 14 (73%) exhibited moderate expression.

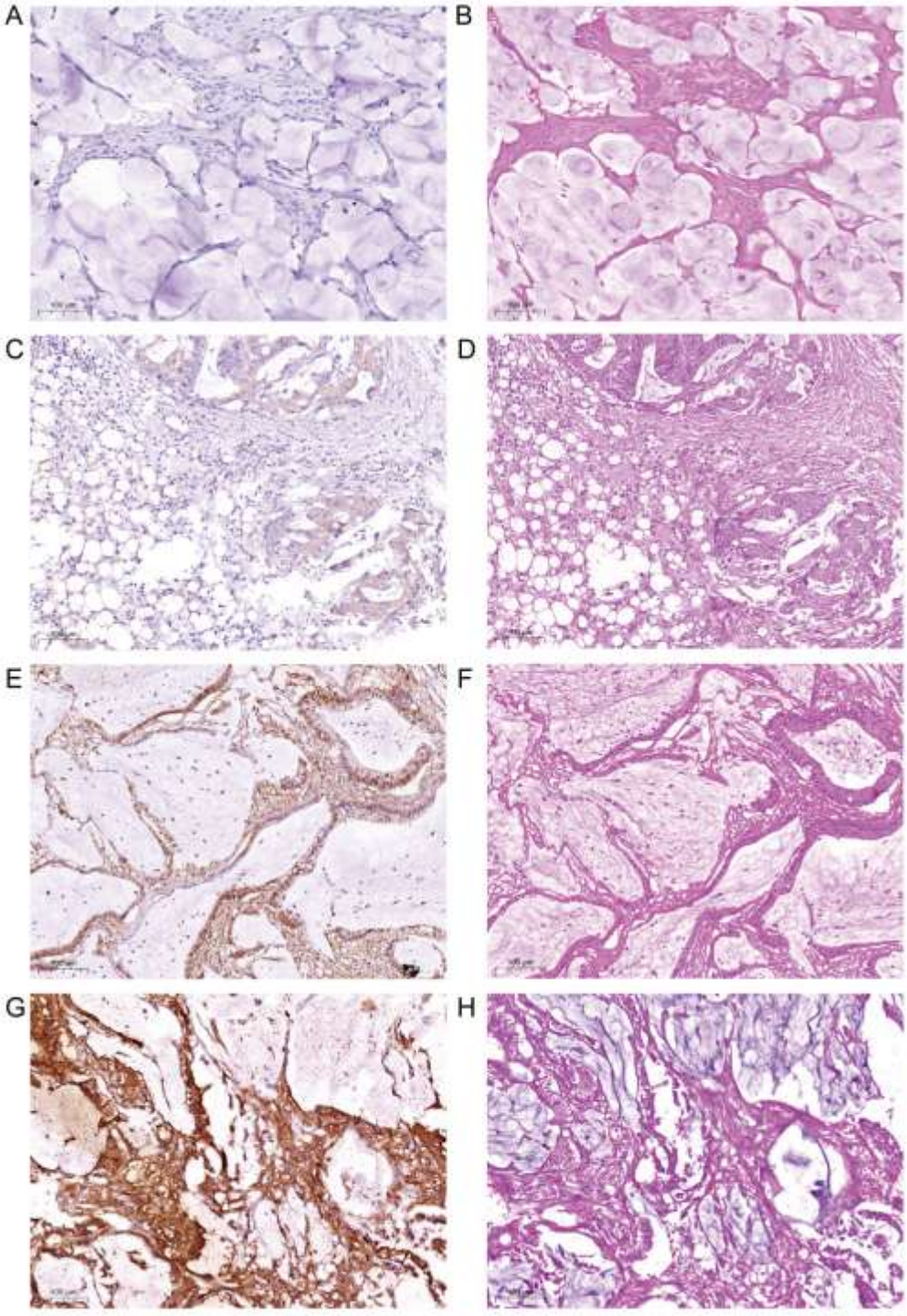


**Figure 1. Staining intensity of claudin 18.2 in colorectal mucinous adenocarcinoma. A and B, C and D, E and F, and G and H are the corresponding areas of immunohistochemical HE staining and of the same lesion. The intensity of positivity was divided into 4 grades: 0 (A), 1(C), 2 (E), and 3 (G).**

### *Expression Differences According to Clinicopathological Features*

The entire cohort and comparison between CLDN18.2-positive and CLDN18.2-negative MACs are summarised in Table 1. A significant trend was observed in the occurrence of CLDN18.2-positive MACs with perineural invasion ($P$ = 0.0235) and lymphovascular invasion ($P$ = 0.0375) compared with CLDN18.2-negative MACs. However, no statistically significant differences were found between CLDN18.2-positive and CLDN18.2-negative MAC cases in terms of patient age, sex, tumour location, aetiology, AJCC TNM staging, presence of lymph node or distant metastasis, lymphatic vessel invasion, tissue type, or mismatch repair status at diagnosis.

**Table 1.** (*continued*).

| **Clinicopathological characteristics** | **Total (N = 91)** | **Claudin 18.2 expression** | | |
|---|---|---|---|---|
| | | **Positive** | **Negative** | ***P*-value** |
| **Lymphovascular invasion, N (%)** | | | | **0.038** |
| Yes | 11 (12.1) | 5 | 6 | |
| No | 80 (87.9) | 13 | 67 | |
| **dMMR, N (%)** | | | | >0.99 |
| Yes | 6 (6.6) | 1 | 5 | |
| No | 85 (93.4) | 17 | 68 | |

### *CLDN18.2 is Frequently Heterogeneously Expressed in MAC*

In our study, IHC scores of both 3+ and 0 were detected within a single tumour tissue, indicating intratumoural heterogeneity in CLDN18.2 expression. To assess the degree of heterogeneity, we defined intratumoural heterogeneity as the coexistence of strong and negative expression. Specifically, when these expressions were present and accounted for more than 50% of the total area, we classified the tumours as exhibiting strong intratumoural heterogeneity. Our findings revealed that nearly all tumours displayed a range of IHC intensities, with nine tumours (9.7%) meeting the criteria for high intratumoural heterogeneity. We further evaluated the distribution patterns of immunostaining in these heterogeneous tumours, which were predominantly characterised by positive or negative clustering, while scattered manifestations were relatively uncommon. Representative images are shown in Figure 2.

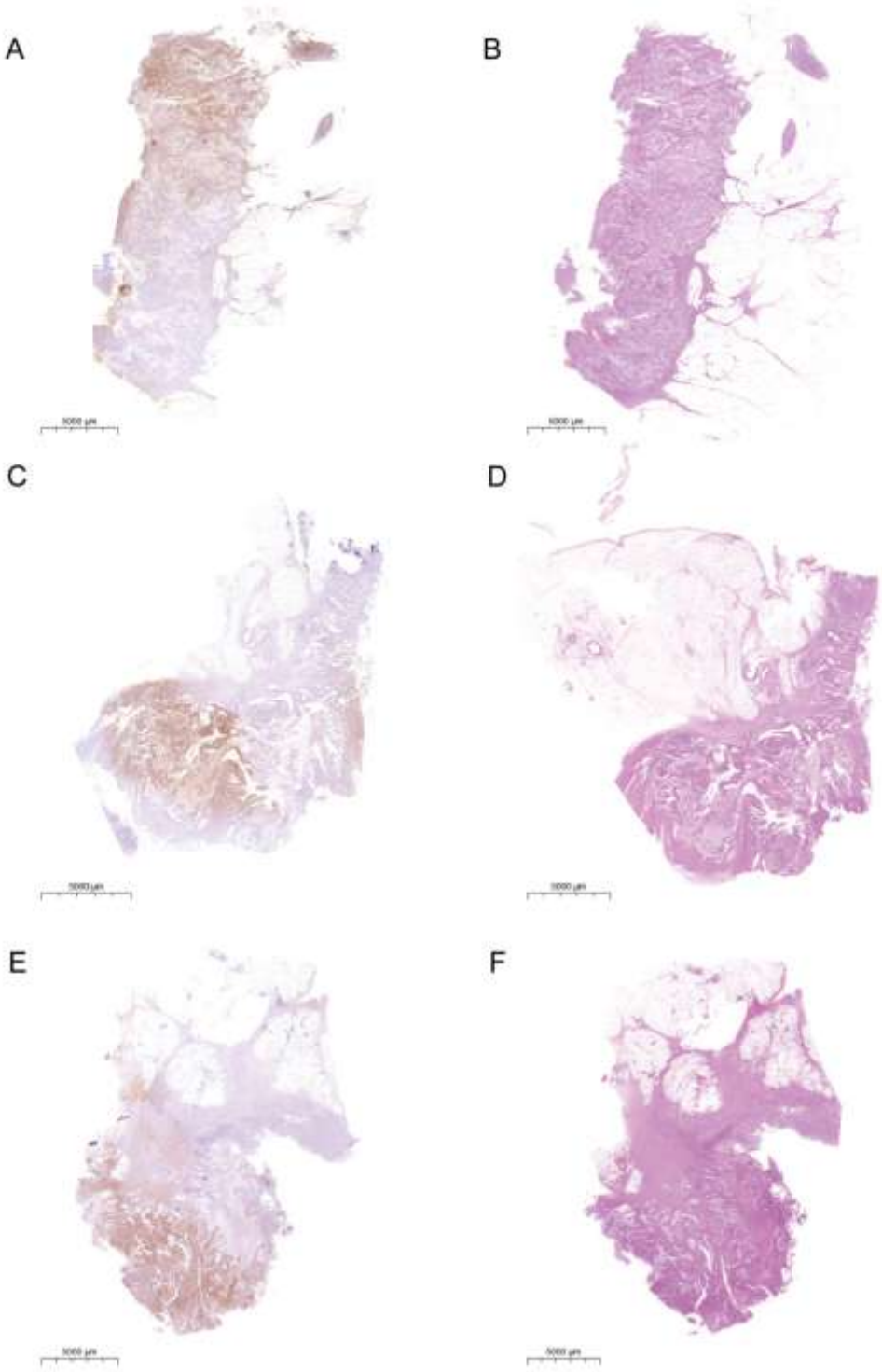


**Figure 2 Staining intensity of claudin 18.2 in colorectal mucinous adenocarcinoma. A and B, C and D, and E and F are the corresponding areas of immunohistochemical HE staining and of the same lesion**

## Discussion

To the best of our knowledge, this is the first study to examine the expression of CLDN18.2 in patients with MAC. Current guidelines primarily focus on the tumour site and molecular phenotype when assisting clinicians in making treatment decisions for CRC, often overlooking the mucinous phenotype[11]. MAC, a distinct subtype of CRC, exhibits resistance to conventional therapies such as radiotherapy and chemotherapy[12]. Moreover, previous studies have established that MAC is associated with a higher propensity for recurrence and metastasis, along

with a substantially poorer prognosis than standard CRA[13]. Therefore, identifying new therapeutic targets for MAC is of utmost importance.

The claudin protein family plays a crucial role as a fundamental component of tight junctions and comprises transmembrane proteins located on the cell membrane[14,15]. CLDN expression has been shown to enhance invasion and metastasis[16]. Claudin 18.2, a member of this family, is encoded by the CLDN18 gene and is typically expressed at low levels in the differentiated epithelial cells of the gastric mucosa, where it primarily maintains intercellular connections[17]. However, the abnormal activation of CLDN18.2 can compromise cell membrane integrity, increase tissue permeability, and promote tumour cell proliferation, differentiation, invasion, and metastasis. Furthermore, CLDN18.2 overexpression may activate relevant signalling pathways, such as PI3K/Akt and MAPK, thereby facilitating tumour cell growth. Under pathological conditions, CLDN18.2 expression is observed in various cancers, including gastric (60%–80%)[18], pancreatic (50%), lung (40%–60%), oesophageal cancers (30%–50%), as well as breast, colon, liver, and ovarian cancers[19]. Thus, CLDN18.2 protein represents a promising target for potential pan-cancer therapies.

Invasive mucinous adenocarcinoma (IMA) is a unique subtype of lung cancer histologically characterised by abundant intracellular mucin[20]. IMA shares similar histological types and clinical features with MAC. Previous studies have confirmed positive CLDN18.2 expression in IMA[20]. CLDN18.2 was found to be associated with histological subtypes, especially the mucinous subtype, as described by Sahin et al. in a pan-cancer analysis[21]. Therefore, we hypothesised that CLDN18.2 may also be positively expressed in other mucinous cancers.

This study is the first to employ IHC to investigate CLDN18.2 expression in a substantial cohort of patients with MAC. Our findings indicated that 18.9% of MAC cases exhibited positive CLDN18.2 expression. Among these, four patients (21%) demonstrated low expression, 14 (73%) exhibited moderate expression, and one (5%) showed high expression. Despite the relatively low positivity rate of CLDN18.2, the absence of specific therapeutic targets for MAC underscores its potential as a novel therapeutic target. A substantial number of patients with MAC may be eligible for CLDN18.2-targeting treatments. Additionally, the commonly observed intratumoural heterogeneity of CLDN18.2 in MAC may pose challenges for diagnostic evaluation.

The relationship between CLDN18.2 and tumor development is still unclear. In this study, we found that CLDN18.2 was associated with perineural and lymphovascular invasion. Vascular structures, lymphatic vessels, and nerves are important routes of metastasis, and perineural and lymphovascular invasion are indicator of poor clinical outcomes and prognosis. The probability of local, regional, or distant recurrence was determined. Therefore, the expression of CLDN18.2 may also be related to tumour progression and recurrence. However, further clinical data are needed to confirm this association.

This study had several limitations. First, it was conducted as a single-centre study with a limited number of tumour samples. Second, we did not explore the association between the CLDN18.2 expression and survival outcomes due to a lack of clinical follow-up data. We plan to collect follow-up data from patients with MACs to investigate the impact on overall survival. Furthermore, the effects of CLDN18.2 on MAC development remain unclear, and the underlying mechanisms require further investigation through *in vivo* and *in vitro* experiments. We intended to collect follow-up data from patients with MAC to assess its impact on overall survival. Additionally, the role of CLDN18.2 in MAC development remains poorly understood, necessitating further exploration of its underlying mechanisms through both *in vivo* and *in vitro* experiments.

## Conclusion

In conclusion, we found that a substantial cohort MAC cases exhibited positive CLDN18.2 expression, suggesting that CLDN18.2 may be a potential therapeutic target of patients with colorectal mucinous adenocarcinoma.

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**Conflict of Interest:** The authors have no relevant financial or non-financial interests to disclose.

**Data Availability Statement:** The data analyzed during the current study are available from the corresponding author on reasonable request.

**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. This work was supported by the Institutional Review Board of Chongqing University Three Gorges Hospital and all the research was performed in accordance with relevant guidelines (No. 2025002).

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