DOI:10.31557/APJCB.2026.11.1.41

RESEARCH ARTICLE

Prognostic Significance of Claudin-4 in Relation to Key Tumor Budding and Invasion Parameters in Colorectal Adenocarcinoma

Ririn Endah Puspitasari[1], Upik Anderiani Miskad[1,2], Muhammad Husni Cangara[1,2], Syarifuddin Wahid[1,2], Djumadi Achmad[1,2], Amalia Yamin[1,3], Suryani Tawali[4]

[1]Department of Anatomical Pathology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia. [2]Department of Anatomical Pathology, Hasanuddin University Hospital, Makassar, Indonesia. [3]Department of Anatomical Pathology, dr. Wahidin Sudirohusodo Hospital, Makassar, Indonesia. [4]Department of Public Health, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.

Abstract

Objective: Colorectal cancer (CRC) is a major global health burden. Claudin-4, a tight junction protein preserving epithelial cohesion, has paradoxically been implicated in tumor progression. Its prognostic role in CRC remains unclear, particularly regarding histopathological grade, tumor budding, tumor-infiltrating lymphocytes (TILs), and deep invasion. This study aimed to clarify these associations. **Methods:** This cross-sectional study included 95 formalin-fixed, paraffin-embedded colorectal adenocarcinoma specimens obtained from 2022 to 2024. Claudin-4 expression was assessed immunohistochemically using the TIS. Tumor budding was evaluated according to ITBCC 2016, TILs based on ITWG criteria, histopathological grade using the WHO classification, and deep invasion (pT1–pT3) based on the AJCC 8th edition. Statistical analysis was performed with Chi-square, Mann–Whitney, and Kruskal–Wallis tests. **Results:** Strong Claudin-4 expression was found in 47.4% of cases. Expression correlated significantly with tumor budding ($p = 0.038$) and deep invasion ($p = 0.038$), but not with histopathological grade ($p = 0.113$) or TILs ($p = 0.170$). Mean TIS values showed a non-significant upward trend with grade and TIL density. **Conclusion:** Claudin-4 is significantly associated with markers of invasiveness, supporting its value as a prognostic biomarker of tumor aggressiveness in colorectal adenocarcinoma.

Keywords: Claudin-4- Colorectal Adenocarcinoma- Tumor Budding- Deep Invasion- Prognostic Marker

Asian Pac J Cancer Biol, **11 (1)**, 41-48

Submission Date: 09/10/2025 Acceptance Date: 12/01/2025

Introduction

Colorectal cancer (CRC) remains a major global health burden. According to GLOBOCAN 2022, it is the third most commonly diagnosed cancer worldwide with 1.9 million new cases (9.6% of all cancers) and the second leading cause of cancer-related mortality, accounting for approximately 904,000 deaths (9.3%) [1-4]. In Indonesia, CRC ranks second among men after lung cancer and third among women after breast and cervical cancers [1, 5]. Despite advances in surgery, chemotherapy, and radiotherapy, standard therapies often fail to eradicate cancer cells and are associated with a high risk of recurrence [6]. Well-established risk factors include older age, prior CRC history, inflammatory bowel disease, sedentary lifestyle, obesity, high red meat intake, smoking, and alcohol consumption [2, 7, 8].

Molecularly, CRC develops through three main pathways: chromosomal instability (CIN), microsatellite instability (MSI), and CpG island methylator phenotype (CIMP) [2, 8-10]. CIN, present in most cases, involves chromosomal imbalances and mutations in APC, KRAS, PI3K, and TP53. MSI results from mismatch repair defects, while CIMP is associated with promoter hypermethylation and silencing of tumor suppressor genes. These mechanisms illustrate how genetic and epigenetic

Corresponding Author:
Prof. Upik Anderiani Miskad
Department of Anatomical Pathology, Faculty of Medicine, Hasanuddin University, Makassar, Indonesia.
Email: upik.miskad@gmail.com

alterations jointly drive colorectal carcinogenesis [2, 8-10].

Prognosis in CRC is traditionally determined by clinicopathological parameters. Histological type, most commonly adenocarcinoma, remains central, while grading based on WHO 2019 criteria distinguishes low grade (≥50% gland formation) from high grade (<50%), with higher grade associated with worse outcomes [3, 9, 11, 12]. Deep invasion, ranging from pTis to pT4, is also pivotal for pathological staging. Tumors confined to the lamina propria are classified as pTis; invasion into the submucosa corresponds to pT1; extension into the muscularis propria is designated as pT2; penetration into the subserosal layer or nonperitonealized pericolic/perirectal tissue is categorized as pT3; and tumors that perforate the visceral peritoneum or directly invade adjacent organs or structures are staged as pT4 [3, 13]. In addition, tumor budding (TB) defined as isolated single cells or clusters of fewer than five cells at the invasive front) is a strong prognostic marker reflecting epithelial mesenchymal transition (EMT). High TB correlates with lymph node metastasis, advanced stage, and poor prognosis [11, 12, 14, 15]. The International Tumor Budding Consensus Conference (ITBCC 2016) standardized TB evaluation using H&E slides and a three-tier system (Bd1: 0–4 buds, Bd2: 5–9 buds, Bd3: ≥10 buds) [16]. Another prognostic factor, TILs, reflects host immune response against tumors [17, 18]. High TIL density generally indicates better outcomes, although routine assessment may be subjective [3, 16-18]. EMT further modulates immune escape, with tumor cells reducing E-cadherin and upregulating PD-L1 to suppress cytotoxic T cells [19-21].

Claudin-4, a critical tight junction protein, regulates epithelial barrier function and maintains tissue cohesion [22-25]. Its expression is frequently dysregulated in CRC and other epithelial cancers. Reduced Claudin-4 promotes EMT, tumor cell motility, and invasiveness, while overexpression may strengthen epithelial barriers, restrict immune infiltration, and enhance angiogenesis [24, 26-28]. Experimental studies demonstrate that Claudin-4 loss is associated with decreased E-cadherin, increased N-cadherin, and activation of PI3K/Akt and Wnt/β-catenin signaling, all of which reinforce EMT (24, 26-28). Dysregulated Claudin-4 expression has also been correlated with histological grade, TB, and TIL density in CRC [22, 24, 27, 29-31]. Collectively, these findings suggest that Claudin-4 plays a paradoxical role in colorectal carcinogenesis: while essential for epithelial stability, its dysregulation contributes to tumor progression and immune modulation.

However, current evidence regarding the prognostic implications of Claudin-4 in CRC remains limited and inconsistent. Some studies emphasize its role as an EMT inhibitor, while others highlight its contribution to tumor progression, angiogenesis, and immune evasion. This duality underscores the need for systematic evaluation across multiple prognostic parameters.

Considering the accumulating evidence that Claudin-4 plays a multifaceted role in the progression of colorectal cancer, we proposed that reduced expression of this protein could be associated with a higher histopathological grade, increased TB activity, lower density of TILs, and deeper tumor invasion. To examine this hypothesis, we carried out a detailed evaluation of Claudin-4 expression in relation to these major morphological and prognostic indicators in colorectal adenocarcinoma. Guided by these considerations this study was designed to validate and refine the associations between Claudin-4 expression and key prognostic histopathological parameters using standardized scoring criteria and a defined clinical cohort. This validation within an Indonesian cohort adds region-specific evidence to the global understanding of Claudin-4 behavior in CRC.

Materials and Methods

Study Design and Samples

This cross-sectional study was conducted at the Laboratory of Anatomical Pathology, Faculty of Medicine, Hasanuddin University, Makassar, using 95 paraffin block samples of histologically confirmed colorectal adenocarcinoma collected from Dr. Wahidin Sudirohusodo General Hospital and Hasanuddin University Hospital between January 2022 and December 2024.

Immunohistochemistry (IHC) and Claudin-4 Expression Assessment

Claudin-4 expression was evaluated by immunohistochemistry (IHC) using rabbit polyclonal Claudin-4 antibody (E-AB-15673, Elabscience; 1:400 dilution). Sections (3 μm) were deparaffinized, stained, and assessed independently by two blinded pathologists. Membranous and/or cytoplasmic staining was examined at 400× magnification. In this study, normal colonic mucosa was used as the positive control, while smooth muscle tissue from the myometrium served as the negative control. Expression was scored semi-quantitatively using the Total Immunostaining Score (TIS), derived from intensity (0–3) and proportion (0–3) scores. The intensity score was categorized as follows: 0/negative (no staining), +1 (weak staining), +2 (moderate staining), and +3 (strong staining). The proportion score was categorized as follows: 0 (0-<5% stained), 1 (5-25% stained), 2 (26-50% stained), and 3 (≥51% stained). Claudin-4 expression was classified as weak (<3), moderate (3–6), or strong (6–9).

Assessment of Clinicopathological Characteristics

Clinicopathological characteristics of the samples, including age, sex, tumor location (proximal, distal, rectum, rectosigmoid), histopathological grade (low-grade and high-grade), TB, TILs, and deep invasion (pT). Histopathological grade was determined by gland formation (low grade ≥50%; high grade <50%). TB was assessed at the invasive front according to ITBCC criteria (Low: 0–4 buds, intermediate: 5–9 buds, high: ≥10 buds per 0.785 mm²). TILs were evaluated histologically and classified per ITWG guidelines as low (0–10%), intermediate (15–50%), or high (55–100%). For the purpose of this study, tumor deep invasion was classified into three categories: T1, defined as invasion limited

to the submucosa; T2, defined as invasion extending through the muscularis propria; and T3, defined as invasion penetrating beyond the muscularis propria into the pericolorectal tissues.

Statistical Analysis

Statistical analyses were conducted using SPSS version 30. Descriptive statistics were applied to summarize the baseline characteristics of the samples, presented as frequencies and distribution tables. Bivariate analyses were performed using the Chi-square test for categorical variables, while the Mann–Whitney and Kruskal–Wallis tests were employed to assess the associations between Claudin-4 expression and continuous or ordinal clinicopathological parameters. A p-value of <0.05 was considered statistically significant.

Results

Claudin-4 immunohistochemical staining showed variable intensity on the membrane and/or cytoplasm of tumor cells. Figure 1 displays representative staining patterns by intensity, while Figure 2 shows tumor budding grades in colorectal adenocarcinoma.

A total of 95 colorectal adenocarcinoma samples were analyzed with diverse clinicopathological characteristics is summarized in Table 1. The age distribution showed that the majority of patients were older than 50 years, accounting for 70 cases (73.7%), while 25 cases (26.3%) were 50 years or younger. With respect to sex, the disease was more frequently found in males (53.7%) compared to females (46.3%). Regarding tumor location, the rectum represented the most common site (35.8%), followed by the proximal colon (29.5%), distal colon (28.4%), and rectosigmoid (6.3%). In terms of histopathological grade, most cases were categorized as low grade (57 cases, 60.0%), whereas 38 cases (40.0%) were classified as high grade. TB analysis revealed 23 cases (24.2%) in the low category, 39 cases (41.1%) in the intermediate category, and 33 cases (34.7%) in the high category. The distribution of TILs showed 44 cases (46.3%) with low TILs, 39 cases (41.1%) with intermediate TILs, and 12 cases (12.6%) with high TILs. Assessment of the depth of tumor invasion revealed that 19 cases (20.0%) were classified as pT1, 36 cases (37.9%) as pT2, and 40 cases (42.1%) as pT3. Claudin-4 expression exhibited a relatively even distribution, with strong expression observed in 45 cases (47.4%), moderate expression in 30 cases (31.6%), and weak expression in 20 cases (21.1%).

Table 1. Clinicopathology Characteristics of the Colorectal Adenocarcinoma Samples

Characteristics	n (%)
Age	
≤50	25 (26.3)
>50	70 (73.7)
Sex	
Male	51 (53.7)
Female	44 (46.3)
Tumor Location	
Proximal	28 (29.5)
Distal	27 (28.4)
Rectum	34 (35.8)
Rectosigmoid	6 (6.3)
Histopathological Grade	
Low	57 (60.0)
High	38 (40.0)
Tumor Budding	
Low	23 (24.2)
Intermediate	39 (41.1)
High	33 (34.7)
TILs	
Low	44 (46.3)
Intermediate	39 (41.1)
High	12 (12.6)
Deep Invasion (pT)	
pT1	19 (20.0)
pT2	36 (37.9)
pT3	40 (42.1)
Claudin-4 Expression	
Strong	45 (47.4)
Moderate	30 (31.6)
Weak	20 (21.1)
Total	95 (100)

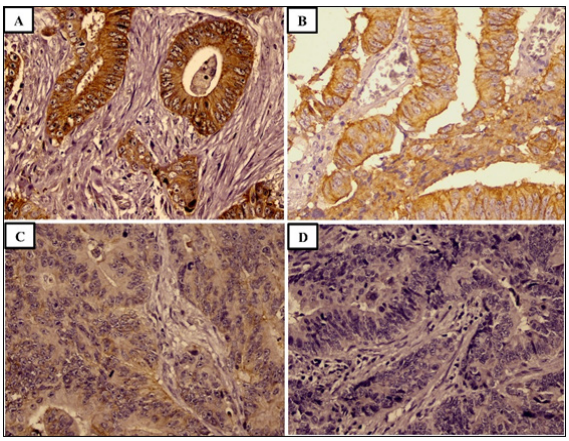

Figure 1. Claudin-4 intensity in colorectal adenocarcinoma. A: Strong (+3); B: Moderate (+2); C: Weak (+1); D: Negative (0). (400x Magnification)

The relationship between Claudin-4 expression and clinicopathological characteristics of colorectal adenocarcinoma is summarized in Table 2. When analyzed according to histopathological grade, low-grade adenocarcinoma showed predominantly moderate Claudin-4 expression (47.4%), whereas strong expression was more frequent in high-grade tumors (34.2%). Although high-grade tumors tended to exhibit higher Claudin-4 expression, the association did not reach statistical significance (p = 0.113). Similarly, the TIS Claudin-4 score was higher in the high-grade group compared to the low-grade group (6.13 ± 2.42 vs. 5.30 ±

Table 2. The relationship between Claudin-4 Expression and Histopathological Grade, Tumor Budding, TILs, and Deep Invasion of Colorectal Adenocarcinoma Samples

Characteristics	Ekspresi Claudin-4			p-Value[a]	TIS Claudin-4	p-Value
	Weak	Moderate	Strong		Mean ± SD	
	n (%)	n (%)	n (%)			
Histopathological Grade						
Low	16 (28.1)	27 (47.4)	14 (24.6)	0.113	5.30 ± 2.78	0.150[b]
High	4 (10.5)	21 (55.3)	13 (34.2)		6.13 ± 2.42	
Tumor Budding						
Low	10 (43.5)	10 (43.5)	3 (13.0)	0.038*	4.39 ± 2.71	0.262[c]
Intermediate	5 (12.8)	21 (53.8)	13 (33.3)		6.08 ± 2.51	
High	5 (15.2)	17 (51.5)	11 (33.3)		5.97 ± 2.61	
TILs						
Low	12 (27.3)	23 (52.3)	9 (20.5)	0.170	5.09 ± 2.62	0.096[c]
Intermediate	8 (20.5)	19 (48.7)	12 (30.8)		5.87 ± 2.75	
High	0 (0.0)	6 (50.0)	6 (50.0)		6.83 ± 2.13	
Deep Invasion (pT)						
pT1	5 (26.3)	9 (47.4)	5 (26.3)	0.038*	5.26 ± 2.56	0.573[c]
pT2	2 (5.6)	24 (66.7)	10 (27.8)		6.31 ± 2.36	
pT3	13 (32.5)	15 (37.5)	12 (30.0)		5.20 ± 2.88	

[a]Analyzed with Chi-square; [b]Analyzed with Mann-Whitney; [c]Analyzed with Kruskal-Wallis; *Significant if p<0.05

2.78), but the difference was not statistically significant (p = 0.150).

With respect to TB, strong Claudin-4 expression was more frequently observed in intermediate (33.3%) and high budding groups (33.3%) compared to the low budding group (13.0%). This difference was statistically significant (p = 0.038). However, the mean TIS Claudin-4 score did not differ significantly across budding categories (p = 0.262).

In terms of TILs, the majority of low TILs cases exhibited moderate expression (52.3%), while strong expression was more common in the intermediate and high TILs groups (20.0% and 50.0%, respectively). Nevertheless, the association was not statistically significant (p = 0.170). The mean TIS Claudin-4 score tended to increase from low to high TILs groups (5.09 ± 2.62; 5.87 ± 2.75; 6.83 ± 2.13), but without statistical significance (p = 0.096).

With respect to tumor invasion depth, although strong Claudin-4 expression was observed slightly more often in advanced lesions (30.0% in pT3) compared with early-stage tumors (26.3% in pT1 and 22.2% in pT2), the numerical variation remained modest. Thus, despite the statistical significance (p < 0.05), this observation should be interpreted prudently, as it may not reflect a biologically meaningful gradient. Furthermore, the mean Claudin-4 tissue immunostaining (TIS) score demonstrated no significant difference among the different pathological stages (p = 0.573), suggesting a limited association between expression intensity and invasion depth.

Discussion

This study analyzed Claudin-4 expression in 95 colorectal adenocarcinoma cases and evaluated its association with key clinicopathological parameters. Claudin-4 immunohistochemical staining showed variable intensities localized to both the membrane and/or cytoplasm of tumor cells, consistent with previous reports that subcellular localization of Claudin-4 may influence its biological function in cancer progression.

This study showed that Claudin-4 expression was not significantly associated with the histopathological grade of colorectal adenocarcinoma. Low expression was more frequent in low-grade tumors, whereas moderate and strong expression were relatively more common in high-grade tumors, but without statistical significance. This aligns with previous studies suggesting that Claudin-4 is more closely related to invasive behavior via the EMT rather than to glandular architecture, which forms the basis of grading [22, 32]. Therefore, although histopathological grade remains a classical prognostic predictor in colorectal cancer, Claudin-4 expression appears to better reflect the biological dynamics of cells at the invasive front rather than merely the degree of morphological differentiation [33].

A significant association was observed between Claudin-4 expression and TB. Interestingly, this study found that high Claudin-4 expression was more frequently observed in cases with high tumor budding activity. This finding contrasts with the initial hypothesis, which assumed that decreased Claudin-4 expression would correlate with increased tumor budding, in line with the theory that loss of tight junction integrity promotes EMT and enhances tumor aggressiveness. However, several underlying biological mechanisms may account for this

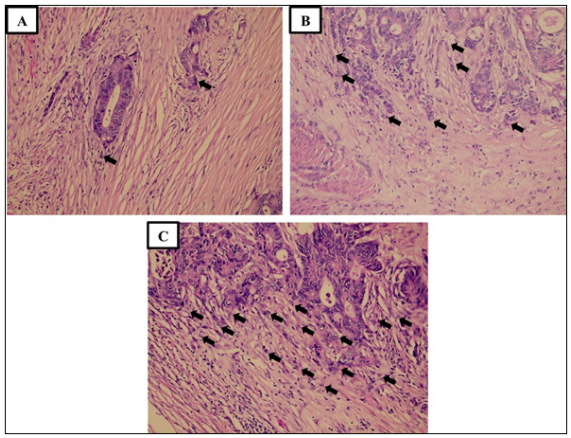

Figure 2. Tumor Budding Grade in Colorectal Adenocarcinoma. A: Low (score 1) ; B: Intermediate (score 2); C: High (score 3). (200x Magnification)

paradoxical phenomenon.

The biological function of Claudin-4 is highly influenced by its subcellular localization. In normal colonic epithelium, Claudin-4 is predominantly restricted to the cell membrane, where it forms a key structural component of tight junctions responsible for maintaining epithelial integrity and intercellular adhesion. This membranous localization prevents uncontrolled cellular migration. In contrast, malignant transformation is frequently accompanied by a distinct redistribution of Claudin-4 from the membrane to the cytoplasm, a phenomenon that compromises epithelial cohesion and promotes stromal invasion an early event in TB [27].

Cytoplasmic accumulation of Claudin-4 has been associated with increased cell motility, resistance to apoptosis, and enhanced invasive potential. Both Osanai et al. [27] and Lin et al. [26] demonstrated that aberrant cytoplasmic localization of Claudin-4 can activate EMT pathways and proteolytic mechanisms that facilitate tissue infiltration. As a result, tumors displaying predominantly cytoplasmic Claudin-4 staining often exhibit a more aggressive and invasive phenotype, despite showing high overall expression by immunohistochemistry. Consistent with this, Fujiwara-Tani et al. [25] reported that cytoplasmic Claudin-4 expression in epithelial malignancies, including CRC, is strongly correlated with deeper invasion and poor clinical outcomes. Thus, accurate immunohistochemical assessment should distinguish membranous from cytoplasmic expression, as the latter represents a critical marker of tumor aggressiveness [24, 26, 27].

Beyond the aspect of subcellular redistribution, the role of Claudin-4 in promoting tumor aggressiveness may also be explained by its involvement in the activation of molecular pathways associated with matrix metalloproteinases (MMPs), particularly MMP-2 and MMP-9. Experimental studies have demonstrated that Claudin-4 overexpression can enhance both the expression and enzymatic activity of MMP-2 and MMP-9, which serve as key mediators in the degradation of the extracellular matrix (ECM). This matrix degradation is a critical step in tumor invasion, as it enables cancer cells to breach the basement membrane and infiltrate adjacent tissues, thereby facilitating invasion and metastasis [34-36].

The overexpression of Claudin-4 within tight junctions may not only enhance intercellular cohesion but also create a physical barrier within the tumor microenvironment that limits the diffusion of various molecules and metabolic products. This restricted diffusion can lead to the local accumulation of pro-tumorigenic factors such as VEGF, IL-8, and lactate within the tumor tissue. The buildup of these factors contributes to the development of a more acidic microenvironment, as the predominantly anaerobic metabolism of cancer cells produces excess lactate [24].

This localized acidity, in turn, stimulates the activation of EMT pathways particularly those involving the Hippo/YAP1 signaling cascade and promotes the loss of epithelial polarity, encouraging a shift toward a more mesenchymal, migratory, and invasive phenotype. Furthermore, the accumulation of pro-tumorigenic mediators and the altered pH of the tumor milieu have been shown to facilitate immune evasion by impairing the infiltration of effector immune cells and upregulating immune checkpoint molecules such as PD-L1, thereby allowing tumor cells to escape immune surveillance [24]. Collectively, these effects reinforce tumor cell migration, invasion, and the formation of tumor budding, ultimately contributing to a poorer prognosis in CRC [24, 37].

Beyond molecular mechanisms and microenvironmental influences, the role of Claudin-4 in colorectal cancer is also profoundly shaped by its biological context. In certain settings, high Claudin-4 expression may support the formation of more cohesive tumor budding clusters, in which small groups of tumor cells retain intercellular adhesion through Claudin-4–mediated tight junctions. This mechanism enables tumor cell clusters to survive environmental stress, enhances their viability during invasion, and facilitates collective migration or an increased potential for metastasis [24, 30]. Moreover, Claudin-4 overexpression may represent an adaptive or compensatory cellular response to invasive stressors such as hypoxia, mechanical compression, or immune pressure at the invasive front. Under such conditions, tumor cells may transiently upregulate Claudin-4 expression to reinforce intercellular cohesion, allowing clusters to persist within an unstable and hostile microenvironment [24].

Taken together, these findings suggest a biphasic model in which Claudin-4 plays distinct, context-dependent roles during tumor progression. In the early phases of colorectal tumorigenesis, membranous Claudin-4 appears to safeguard epithelial structure and maintain intercellular cohesion. However, as the disease advances, the abnormal relocation of Claudin-4 into the cytoplasm seems to facilitate tissue invasion, coordinated cell movement, and the acquisition of metastatic traits. This perspective offers a plausible explanation for the paradox that elevated Claudin-4 expression may accompany, rather than oppose, aggressive tumor behavior in CRC.

These observations underscore the context-dependent nature of Claudin-4 function it may play a protective role in maintaining cell integrity under certain conditions,

yet in others, it can paradoxically enhance the invasive capacity and survival of tumor cell clusters [24, 30]. Therefore, interpretation of Claudin-4 expression in CRC should take into account not only its expression level but also its subcellular localization and the molecular context of the tumor. Further studies integrating subcellular localization assessment with molecular analyses are warranted to provide deeper insight into the multifaceted role of Claudin-4 in CRC progression.

Claudin-4 expression was not significantly associated with TILs, although higher immunoreactivity scores were observed in moderate and high TIL groups. Abnormal Claudin-4 may reinforce epithelial barriers and hinder effector immune infiltration [29]. Recent studies suggest that Claudin-4 can also modulate tumor–immune interactions via EMT pathways and immune checkpoint regulation, including PD-L1 [19, 31]. The absence of statistical significance in this study may reflect inherent variability within the tumor microenvironment, which can influence biomarker expression and immune interactions. Nonetheless, the observed near-significant trend suggests that Claudin-4 potentially plays a role in modulating the tumor immune landscape. This observation strengthens the biological plausibility of Claudin-4 as a relevant factor and underscores the necessity of validating these findings in larger cohorts with integrative molecular approaches.

Claudin-4 expression demonstrated a statistically significant association with the depth of tumor invasion ($p = 0.038$), as higher expression levels were more frequently found in advanced-stage tumors (pT3). This observation implies that Claudin-4 may play a contributory role in promoting deeper stromal infiltration and tumor advancement. Previous studies have shown that altered regulation of Claudin family proteins can disrupt epithelial integrity, thereby facilitating tumor cell dissemination into the surrounding stroma [33, 38]. Interestingly, the lack of significant differences in mean TIS values among stages suggests that Claudin-4's influence may rely less on the absolute expression level and more on its spatial redistribution or subcellular localization [33, 39]. Paradoxically, our findings revealed that tumors with deeper invasion (pT3–pT4) exhibited higher Claudin-4 expression, highlighting its context-dependent behavior in CRC biology. Recent literature further supports this, indicating that aberrant cytoplasmic accumulation of Claudin-4 can paradoxically promote tumor progression by enhancing cellular motility, apoptosis resistance, and metastatic potential [27]. Interestingly, the mean Claudin-4 Tumor Invasion Score (TIS) did not display a consistent linear trend across stages, as intermediate-stage tumors (pT2) exhibited slightly higher scores than either early (pT1) or advanced (pT3) lesions. Such variability suggests that Claudin-4's role in invasion may rely less on expression magnitude and more on subcellular redistribution or context-specific activation. Consistent with this view, recent findings indicate that cytoplasmic mislocalization of Claudin-4 can paradoxically enhance cell motility, confer resistance to apoptosis, and increase metastatic capacity [24]. Collectively, these observations highlight the complex, context-dependent function of Claudin-4 within colorectal tumor biology.

This study has certain limitations. It did not include molecular analyses such as gene expression or methylation profiling, which could clarify mechanisms underlying Claudin-4 dysregulation. Survival data were also unavailable, preventing direct evaluation of its prognostic value alongside budding and TILs. Furthermore, microenvironmental factors such as stromal composition and immune checkpoint markers were not assessed. Hence, the findings should be interpreted with caution and validated in larger multidimensional studies.

In conclusion, this study does not introduce a completely new concept but confirms and refines previous findings about the role of Claudin-4 in colorectal adenocarcinoma. Our results show that changes in Claudin-4 expression are closely related to tumor invasiveness, supporting its potential use as an additional prognostic biomarker. By applying standardized histopathological criteria in a defined patient cohort, this study adds clearer evidence to the understanding of Claudin-4's role in tumor progression. These findings also highlight the potential of incorporating Claudin-4 into future biomarker panels to improve prognostic accuracy and support more personalized treatment decisions.

Acknowledgements

General

The authors thank the staff of the Anatomical Pathology Laboratory, Universitas Hasanuddin Hospital and Dr. Wahidin Sudirohusodo Hospital, Makassar, for their technical and logistical support.

Funding Statement

This research received some funding/ support from Immuno-Oncology, Thematic Research Group Hasanuddin University, No15523/UN4.1/KEP/2024.

Approval

This work was permitted by the research committee of the Faculty of Medicine, Hasanuddin University.

Conflict of Interest

The authors declare no competing interests.

Ethical Declaration

The Ethics Committee of the Faculty of Medicine has given approval for this study (Protocol # UH24A9AV23 – Registry 905/UN4.6.4.5.31/ PP36/ 2A24)

Authors' Contributions

Data Availability

On reasonable request, the associated author will release the datasets used in this work.

Study Registration

Not applicable. This study was a retrospective cross-sectional analysis using archived paraffin-embedded specimens and therefore was not registered in a clinical

trial registry.

Originality Declaration for Figures

All figures included in this manuscript are original and have been created by the authors specifically for the purposes of this study. No previously published or copyrighted images have been used. The authors confirm that all graphical elements, illustrations, and visual materials were generated from the data obtained in the course of this research or designed uniquely for this manuscript.

References

1. Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, Jemal A. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA: a cancer journal for clinicians. 2024;74(3):229-263. https://doi.org/10.3322/caac.21834
2. Mármol I, Sánchez-de-Diego C, Pradilla Dieste A, Cerrada E, Rodriguez Yoldi MJ. Colorectal Carcinoma: A General Overview and Future Perspectives in Colorectal Cancer. International Journal of Molecular Sciences. 2017 01 19;18(1):197. https://doi.org/10.3390/ijms18010197
3. Rianti AM, Cangara MH, Yamin A, Dahlan H, Ilyasa MR, Miskad UA. FGFR2 as A Prognostic and Predictive Marker in Colorectal Adenocarcinoma Based on TILs Grade. The Indonesian Biomedical Journal. 2025 04 01;17(2):188-96. https://doi.org/10.18585/inabj.v17i2.3523
4. Miskad UA, Henry MM, Pardamean CI, Budiarto A, Irwan A, Baurley JW, Yusuf I, Pardamean B. Colorectal cancer in south sulawesi: a case-control study for nongenetic risk factors. Oncology Reviews. 2025 05 30;19. https://doi.org/10.3389/or.2025.1589655
5. Harjanti DA, Murtono C, Suryawinata K, Halim A, Wiryokusuma MF, Djitro TB. John Cunningham Virus T-Antigen Expression on Mild and Severe Dysplasia Adenomatous Polyp, Low and High Grade Adenocarcinoma of The Colon. Molecular and Cellular Biomedical Sciences. 2019 03 01;3(1):7-12. https://doi.org/10.21705/mcbs.v3i1.36
6. Wulandari F, Hanifa M. Integrative Bioinformatics Reveals the Lactate Dehydrogenase B (LDHB) Significance in Colon Adenocarcinoma. Molecular and Cellular Biomedical Sciences. 2023 07 24;7(2):98-108. https://doi.org/10.21705/mcbs.v7i2.346
7. Sawicki T, Ruszkowska M, Danielewicz A, Niedźwiedzka E, Arłukowicz T, Przybyłowicz KE. A Review of Colorectal Cancer in Terms of Epidemiology, Risk Factors, Development, Symptoms and Diagnosis. Cancers. 2021 04 22;13(9):2025. https://doi.org/10.3390/cancers13092025
8. Duan B, Zhao Y, Bai J, Wang J, Duan X, Luo X, Zhang R, Pu Y, Kou M, Lei J, Yang S. Colorectal Cancer: An Overview. Gastrointestinal Cancers. 2022.
9. Nefedova NA, Kharlova OA, Malkov PG. Expression of claudin-1, 3, and 4 in colorectal cancer and polyps. Arkhiv Patologii. 2016;78(3):11-19. https://doi.org/10.17116/patol201678311-19
10. Alzahrani SM, Al Doghaither HA, Al-Ghafari AB. General insight into cancer: An overview of colorectal cancer (Review). Molecular and Clinical Oncology. 2021 Dec;15(6):271. https://doi.org/10.3892/mco.2021.2433
11. Mehta A, Goswami M, Sinha R, Dogra A. Histopathological Significance and Prognostic Impact of Tumor Budding in Colorectal Cancer. Asian Pacific journal of cancer prevention: APJCP. 2018 09 26;19(9):2447-2453. https://doi.org/10.22034/APJCP.2018.19.9.2447
12. Qu Q, Wu D, Li Z, Yin H. Tumor budding and the prognosis of patients with metastatic colorectal cancer: a meta-analysis. International Journal of Colorectal Disease. 2023 05 24;38(1):141. https://doi.org/10.1007/s00384-023-04423-8
13. Theodora I, Sudiana IK, Budipramana VS, Erwin F, Dewi S, Novita BD .Tumor Differentiation is Correlated with Estrogen Receptor Beta (ERβ) Expression but Not with Interleukin-6 (IL-6) Expression in Colorectal Carcinoma. Indones Biomed J. 2024;16(5):481–6. https://doi.org/10.18585/inabj.v16i5.3324
14. Hutasoit GA, Miskad UA, Akil F, Cangara MH, Dahlan H, Yamin A, Mardiati M. Snail Expression as a Prognostic Factor in Colorectal Adenocarcinoma. Asian Pacific journal of cancer prevention: APJCP. 2024 09 01;25(9):3143-3149. https://doi.org/10.31557/APJCP.2024.25.9.3143
15. Kim WS, Kim H, Joo MK, Choi BI, Yoo AY, Park J, Lee BJ, Kim SH, Chun HJ. High Expression of Claudin-4 Is Associated with Synchronous Tumors in Patients with Early Gastric Cancer. Journal of Clinical Medicine. 2022 06 20;11(12):3550. https://doi.org/10.3390/jcm11123550
16. Lugli A, Kirsch R, Ajioka Y, Bosman F, Cathomas G, Dawson H, El Zimaity H, et al. Recommendations for reporting tumor budding in colorectal cancer based on the International Tumor Budding Consensus Conference (ITBCC) 2016. Modern Pathology: An Official Journal of the United States and Canadian Academy of Pathology, Inc. 2017 09;30(9):1299-1311. https://doi.org/10.1038/modpathol.2017.46
17. Piroozkhah M, Gholinezhad Y, Piroozkhah M, Shams E, Nazemalhosseini-Mojarad E. The molecular mechanism of actions and clinical utilities of tumor infiltrating lymphocytes in gastrointestinal cancers: a comprehensive review and future prospects toward personalized medicine. Frontiers in Immunology. 2023;14:1298891. https://doi.org/10.3389/fimmu.2023.1298891
18. Kong JC, Guerra GR, Pham T, Mitchell C, Lynch AC, Warrier SK, Ramsay RG, Heriot AG. Prognostic Impact of Tumor-Infiltrating Lymphocytes in Primary and Metastatic Colorectal Cancer: A Systematic Review and Meta-analysis. Diseases of the Colon and Rectum. 2019 04;62(4):498-508. https://doi.org/10.1097/DCR.0000000000001332
19. Chen L, Gibbons DL, Goswami S, Cortez MA, Ahn Y, Byers LA, Zhang X, et al. Metastasis is regulated via microRNA-200/ZEB1 axis control of tumour cell PD-L1 expression and intratumoral immunosuppression. Nature Communications. 2014 Oct 28;5:5241. https://doi.org/10.1038/ncomms6241
20. Yang J, Antin P, Berx G, Blanpain C, Brabletz T, Bronner M, Campbell K, et al . Guidelines and definitions for research on epithelial-mesenchymal transition. Nature Reviews. Molecular Cell Biology. 2020 06;21(6):341-352. https://doi.org/10.1038/s41580-020-0237-9
21. Terry S, Savagner P, Ortiz-Cuaran S, Mahjoubi L, Saintigny P, Thiery J, Chouaib S. New insights into the role of EMT in tumor immune escape. Molecular Oncology. 2017 07;11(7):824-846. https://doi.org/10.1002/1878-0261.12093
22. Hana C, Thaw Dar NN, Galo Venegas M, Vulfovich M. Claudins in Cancer: A Current and Future Therapeutic Target. International Journal of Molecular Sciences. 2024 04 24;25(9):4634. https://doi.org/10.3390/ijms25094634
23. Bansal P, Choudhary S, Taneja T, Sangwan S, Gupta B, Goyal S, et al. We are IntechOpen , the world ' s leading publisher of Open Access books Built by scientists , for scientists TOP 1 %. Intech [Internet]. 2016;i(tourism):15. Available from: https://www.intechopen.com/books/advanced-biometric-

technologies/liveness-detection-in-biometrics..
24. Fujiwara-Tani R, Mori S, Ogata R, Sasaki R, Ikemoto A, Kishi S, Kondoh M, Kuniyasu H. Claudin-4: A New Molecular Target for Epithelial Cancer Therapy. International Journal of Molecular Sciences. 2023 03 13;24(6):5494. https://doi.org/10.3390/ijms24065494
25. Fujiwara-Tani R, Sasaki T, Luo Y, Goto K, Kawahara I, Nishiguchi Y, Kishi S, et al. Anti-claudin-4 extracellular domain antibody enhances the antitumoral effects of chemotherapeutic and antibody drugs in colorectal cancer. Oncotarget. 2018 Dec 21;9(100):37367-37378. https://doi.org/10.18632/oncotarget.26427
26. Lin X, Shang X, Manorek G, Howell SB. Regulation of the Epithelial-Mesenchymal Transition by Claudin-3 and Claudin-4. PloS One. 2013;8(6):e67496. https://doi.org/10.1371/journal.pone.0067496
27. Osanai M, Murata M, Chiba H, Kojima T, Sawada N. Epigenetic silencing of claudin-6 promotes anchorage-independent growth of breast carcinoma cells. Cancer Science. 2007 Oct;98(10):1557-1562. https://doi.org/10.1111/j.1349-7006.2007.00569.x
28. Li C, Zhang X, Fan G. Updates in colorectal cancer stem cell research. Journal of Cancer Research and Therapeutics. 2014 Dec;10 Suppl:233-239. https://doi.org/10.4103/0973-1482.151449
29. Singh H, Nugent Z, Demers AA, Kliewer EV, Mahmud SM, Bernstein CN. The reduction in colorectal cancer mortality after colonoscopy varies by site of the cancer. Gastroenterology. 2010 Oct;139(4):1128-1137. https://doi.org/10.1053/j.gastro.2010.06.052
30. Li J. Context-Dependent Roles of Claudins in Tumorigenesis. Frontiers in Oncology. 2021;11:676781. https://doi.org/10.3389/fonc.2021.676781
31. Adib E, Jabar F, Tafavvoghi M, Nassar A, Bou Farhat E, Mamon HJ, Ng K, et al. Deep learning–powered analysis of tumor-infiltrating lymphocytes (TILs) in colorectal cancer.. Journal of Clinical Oncology. 2025 02;43(4_suppl):288-288. https://doi.org/10.1200/JCO.2025.43.4_suppl.288
32. Tokuhara Y, Morinishi T, Matsunaga T, Ohsaki H, Kushida Y, Haba R, Hirakawa E. Claudin-1, but not claudin-4, exhibits differential expression patterns between well- to moderately-differentiated and poorly-differentiated gastric adenocarcinoma. Oncology Letters. 2015 07;10(1):93-98. https://doi.org/10.3892/ol.2015.3208
33. Ueda J, Semba S, Chiba H, Sawada N, Seo Y, Kasuga M, Yokozaki H. Heterogeneous expression of claudin-4 in human colorectal cancer: decreased claudin-4 expression at the invasive front correlates cancer invasion and metastasis. Pathobiology: Journal of Immunopathology, Molecular and Cellular Biology. 2007;74(1):32-41. https://doi.org/10.1159/000101049
34. Takehara M, Nishimura T, Mima S, Hoshino T, Mizushima T. Effect of claudin expression on paracellular permeability, migration and invasion of colonic cancer cells. Biological & Pharmaceutical Bulletin. 2009 05;32(5):825-831. https://doi.org/10.1248/bpb.32.825
35. Hwang T, Lee L, Wang C, Liang Y, Huang S, Wu C. Claudin-4 expression is associated with tumor invasion, MMP-2 and MMP-9 expression in gastric cancer. Experimental and Therapeutic Medicine. 2010 09;1(5):789-797. https://doi.org/10.3892/etm.2010.116
36. Fatimah S, Rahaju AS, Rahniayu A. Role of claudin-4 and matrix metalloproteinase-2 in tumor invasion of colorectal adenocarcinoma. Research Journal of Pharmacy and Technology. 2021 09;14(9):4795-4800. https://doi.org/10.52711/0974-360X.2021.00834
37. Estrella V, Chen T, Lloyd M, Wojtkowiak J, Cornnell HH, Ibrahim-Hashim A, Bailey K, et al. Acidity generated by the tumor microenvironment drives local invasion. Cancer Research. 2013 03 01;73(5):1524-1535. https://doi.org/10.1158/0008-5472.CAN-12-2796
38. Swisshelm K, Macek R, Kubbies M. Role of claudins in tumorigenesis. Advanced Drug Delivery Reviews. 2005 04 25;57(6):919-928. https://doi.org/10.1016/j.addr.2005.01.006
39. Hewitt KJ, Agarwal R, Morin PJ. The claudin gene family: expression in normal and neoplastic tissues. BMC cancer. 2006 07 12;6:186. https://doi.org/10.1186/1471-2407-6-186