

THE ASSOCIATION OF C-MET EXPRESSION WITH HISTOLOGICAL SUBTYPE, DEGREE OF DIFFERENTIATION, DEPTH OF INVASION, AND NODAL STATUS IN COLORECTAL ADENOCARCINOMA AT NGOERAH HOSPITAL, DENPASAR, BALI, INDONESIA

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ABSTRACT

Background: Colorectal adenocarcinoma (CRA) exhibits biological heterogeneity that dictates disease aggressiveness and impacts patient prognosis. Although c-MET activation drives tumor progression and metastasis, its prognostic role in CRC research remains variable. The objectives of this study are to demonstrate the association of c-MET expression with histological subtype, degree of differentiation, depth of invasion, and nodal status in colorectal adenocarcinoma at Ngoerah Hospital, Denpasar.

Methods: This analytical observational cross-sectional study utilized 40 archival formalin-fixed paraffin-embedded (FFPE) tissue blocks from patients diagnosed with CRA (January 2023 to March 2026) obtained via consecutive sampling according to specific inclusion and exclusion criteria. c-MET expression was assessed using immunohistochemistry with a semi-quantitative Histo-score (H-Score) scoring method, utilizing a median value of 150 as the cut-off point. Data analysis was performed using the chi-square test, prevalence ratio (PR) calculations with a 95% confidence interval (CI), and multivariate analysis.

Results: High c-MET protein expression was detected in 57.5% of patients. No statistically significant association was observed between c-MET expression and histological subtype ($p=0.893$). However, significant bivariate associations were identified between c-MET expression and the degree of differentiation ($p=0.003$; PR=2.214; 95%CI: 1.502–3.264), depth of invasion ($p=0.006$; PR=2.400; 95%CI: 1.119–5.145), and nodal status ($p=0.002$; PR=2.850; 95%CI: 1.197–6.787). In multivariate analysis, the statistical significance of depth of invasion was lost ($p=0.274$) when tested alongside the degree of differentiation and nodal status. Consequently, nodal status emerged as the strongest independent factor associated with high c-MET expression.

Conclusion: c-MET expression is significantly associated with the degree of differentiation and nodal status, but shows no association with the histological subtype in CRC. Further analysis found that the depth of invasion is not an independent predictor of c-MET expression and nodal status acts as the strongest independent factor.

Keywords: Colorectal adenocarcinoma, c-MET, immunohistochemistry.

INTRODUCTION

Colorectal carcinoma (CRC) is a malignancy that constitutes a major cause of morbidity and mortality worldwide. Colorectal adenocarcinoma (CRA) is a malignant neoplasm originating from the epithelium of the large intestine or rectum, representing the most predominant histopathological type of CRC.

CRA is the third most common type of cancer in the world, accounting for approximately 10% of all cancer cases, and is the second leading cause of cancer-related deaths globally.^{1,2} The highest incidence rates of CRA are found in industrialized regions such as Europe, Australia, New Zealand, and North America, whereas lower rates are observed in South Asian countries such as India, followed by countries on the African continent.³ The majority of CRA cases are found in older age groups, with most occurring in individuals aged 50 years and above.²

In Indonesia, in 2020, there were 389,768 new cancer cases and 232,302 cancer-related deaths. Of these, 34,189 cases (8.8%) were new CRA cases, and 17,786 cases (7.8%) were deaths caused by CRA.³ Data from Negeri Hospital, Denpasar, (2014-2016) showed 75 cases (7.11%) of rectal carcinoma and 38 cases (3.60%) of colon carcinoma.⁴ The prevalence of CRA in Bali is the third highest nationally and exhibits an upward trend.⁵

Traditional biomarkers are biological indicators that have long been used in clinical practice to measure the aggressiveness of a neoplasm. These biomarkers are essential for determining an accurate prognosis and comparing various treatment protocols. Various histopathological biomarkers utilized for this purpose include histological subtype, grading (degree of differentiation), depth of invasion, and nodal status.⁶

The degree of differentiation refers to how closely the parenchymal cells of a neoplasm resemble their cells of origin. Graded differentiation, ranging from well to poorly differentiated, varies depending on the type of malignant neoplasm. In CRA, the degree of differentiation is based on the proportion of glandular structures formed by neoplastic cells. Currently, there are two tiers of differentiation grading in CRA: low-grade and high-grade differentiation.⁷ Low-grade differentiation is a condition where the CRA architecture resembles normal structures, characterized by abundant glandular structures (previously encompassing well to moderate differentiation). High-grade differentiation is an architecture where cancer cells exhibit little or no resemblance to normal tissue structures (formerly termed poorly differentiated). If there are areas with heterogeneous differentiation, the grade is determined based on the area with the least glandular formation (the poorest differentiation). Generally, poorly differentiated tumors tend to be more aggressive. The invasive front, the boundary between the tumor and normal tissue, frequently undergoes epithelial-mesenchymal transition (EMT) changes, known as tumor budding. Although clinically significant, this area is not used to determine grading in CRA. Tumor budding is reported separately from the degree of differentiation.^{6,7}

One of the biological characteristics of cancer cells is their ability to infiltrate surrounding tissues and spread through lymphatic and hematogenous pathways, resulting in carcinomatous metastases. The staging of CRA is determined

using the TNM system. The T category indicates the depth of invasion, N assesses regional lymph node involvement, and M classifies metastasis based on the location and number of organs involved. The TNM classification is crucial for prognosis and treatment planning. The heterogeneity of CRA, particularly in T3 and T1 status, necessitates more detailed stratification for better prognostication. Specifically for T1, substaging has become increasingly important with the rise in population screening cases. The TNM stage, particularly T and N, holds greater clinical value than the histological degree of differentiation in determining prognosis.^{6,7}

It is undeniable that the heterogeneity of CRA distinguishes the biological behavior of one tumor from another, thereby influencing prognosis and therapeutic response. CRA heterogeneity is reflected in variations of histological subtypes (mucinous adenocarcinoma, signet ring cell), degree of differentiation, and staging. The vast majority of colorectal malignancies present as adenocarcinoma NOS (>95%), while other histological subtypes are less frequently encountered.⁸ This heterogeneity creates a demand for biomarkers capable of predicting prognosis more accurately. Historically, CRA has been one of the most extensively studied cancers, leading to the discovery of various biomarkers. Several biomarkers have served as potential prognostic and predictive markers. In 2019, the WHO classified the biomarker groups for CRA. The first group consists of validated biomarkers, such as RAS gene testing. The second group comprises potentially significant biomarkers. Biomarkers in this group may be meaningful based on preliminary studies, but further research is required to validate these findings to ensure their accuracy. DNA, RNA, and protein molecules of c-MET have garnered attention and emerged as one of the biomarkers within this category.⁷

The c-MET receptor tyrosine kinase, encoded by the MET proto-oncogene, interacts with its high-affinity ligand, hepatocyte growth factor (HGF), also known as scatter factor. This receptor was first identified in 1980. Under non-tumorigenic physiological conditions, the HGF/c-MET axis plays a key role in tissue patterning during early embryogenesis, wound healing, and organ regeneration.⁹⁻¹¹ Preclinical studies, including those utilizing cellular models of colorectal, gastric, and gastroesophageal cancers, have demonstrated that the HGF/c-MET pathway is essential for growth, cell survival, invasion, and both intrinsic and acquired drug resistance in gastrointestinal cancers.

Aberrant HGF/c-MET signaling harbors oncogenic potential and is implicated in the development and metastasis of various tumors, including CRA, gastric carcinoma, and Gastroesophageal Junction (GEJ) carcinomas.¹² This oncogenic activation includes MET amplification and mutation, transcriptional deregulation, inadequate c-MET degradation, and abnormal autocrine or paracrine HGF production. Overexpression of c-MET, detected via immunohistochemistry (IHC) and/or fluorescence in situ hybridization (FISH), represents an independent marker for poor prognosis in various solid tumors. In patients with gastric and GEJ carcinomas, c-MET overexpression and/or amplification correlates with a poorer prognosis compared to patients without c-MET overexpression.¹³⁻¹⁶ This remains

consistent across various stages and diagnostic methods, highlighting the critical role of c-MET in the progression of gastric cancer.

Study findings regarding the prognostic role of c-MET in CRA have yielded variable results. Several studies report that the prognostic value of c-MET is limited to stage IV patients.¹⁷⁻²² Experts hypothesize that this inconsistency may be attributed to differences in c-MET expression detection methods (IHC, qRT-PCR, FISH) and the heterogeneity of patient cohorts concerning TNM staging. A study by Liu et al. indicated that high c-MET expression in stage III-IV CRA correlates with poorer survival rates.²³

Research on c-MET in CRA has predominantly been conducted on Caucasian populations. Inter-ethnic variations, encompassing differences in genetic factors, diet, and environmental exposures, theoretically have the capacity to influence biomarker expression; therefore, generalizations of these study results cannot be made directly. The 2023 report by the International Agency for Research on Cancer (IARC) emphasizes the necessity of regional validation for biomarker expression to ensure predictive and prognostic accuracy across different populations or ethnicities. To date, no similar study has been conducted in Bali, Indonesia. Therefore, it is necessary to conduct research on c-MET in CRA at Ngoerah Hospital, Denpasar, to bridge this gap. Consequently, this study is anticipated to provide novel insights into the role of c-MET as a prognostic biomarker in CRA.

The objectives of this study are to demonstrate the association of c-MET expression with histological subtype, degree of differentiation, depth of invasion, and nodal status in colorectal adenocarcinoma at Ngoerah Hospital, Denpasar.

METHODS

Study Design and Sample Selection

This analytical observational study employed a cross-sectional design. The study was conducted at the Department of Anatomical Pathology, Ngoerah Hospital, Denpasar, using archival formalin-fixed paraffin-embedded (FFPE) tissue blocks from patients diagnosed with CRA between January 2023 and March 2026. Furthermore, the clinicopathological data of the patients, encompassing age, sex, histological subtype, degree of differentiation, depth of invasion, and nodal status, were retrieved from the medical record system at Ngoerah Hospital, Denpasar.

The study samples consisted of all CRA patients who underwent histopathological evaluation at the Anatomical Pathology Laboratory of Ngoerah Hospital, Denpasar, during the study period and met the specific inclusion and exclusion criteria. Samples were selected using a consecutive sampling method until the target sample size of 40 samples was achieved.

Immunohistochemistry Staining Procedure

Sections (3 µm thick) were cut using a Leica 2125 RM microtome, mounted on poly-L-lysine-coated Biogear slides, and air-dried for 24 hours. Automated IHC staining was performed using a Leica Bond-Max Immunostainer. The anti-MET rabbit polyclonal antibody (Bioenzy BZ-0847350AF-AP, dilution 1:200) was applied for 30 minutes at room temperature, followed

by incubation with HRP polymer and DAB chromogen. Slides were counterstained with haematoxylin and examined using a Leica DM750 microscope.

Evaluation of c-MET Expression

The interpretation of c-MET staining was performed using a semi-quantitative approach known as the Histo score (H-score) to evaluate each case based on the percentage of stained cells and staining intensity. The staining intensity was graded into the following four categories: 0 (negative); 1+ (weak intensity); 2+ (moderate intensity); and 3+ (strong intensity).²²

The H-score was calculated using the following formula: $1 \times (\% \text{ of } 1+ \text{ cells}) + 2 \times (\% \text{ of } 2+ \text{ cells}) + 3 \times (\% \text{ of } 3+ \text{ cells})$. This calculation yielded an H-score ranging from 0 to 300. The mean or median H-score was selected as the cut-off value to stratify patients into low and high c-MET expression cohorts. The mean value was applied if the data demonstrated a normal distribution, whereas the median was utilized for non-normally distributed data. The evaluation focused on cytoplasmic and cell membrane staining, wherein positively stained cells exhibited a brown.^{15,22}

Positive c-MET protein expression was assessed specifically within tumor cells. Cases of poorly differentiated hepatocellular carcinoma or CRA were utilized as positive controls. Because the c-MET protein is not expressed in inflammatory cells, vascular endothelial cells, smooth muscle cells, or fibroblasts, these cell types served as internal negative controls for each immunohistochemical (IHC) evaluation.²²

Statistical Analysis

Data analysis was performed using the Statistical Package for the Social Sciences (SPSS) software version 26.0 for Windows. Initially, a descriptive analysis was conducted to determine the distribution of research subjects regarding age, sex, histological subtype, degree of differentiation, depth of invasion, and nodal status.

The normality of the H-score data was evaluated using the Shapiro-Wilk test. Specifically, the mean was applied as the cut-off for normally distributed data, while the median was utilized for non-normally distributed data. Based on these normality results, either the mean or median H-score was selected as the cut-off value to categorize patients into low (less than the mean/median) and high (greater than or equal to the mean/median) c-MET expression groups.

Subsequently, a chi-square test was employed to analyze the association between c-MET expression and histological subtype, degree of differentiation, depth of invasion, and nodal status. For variable relationships that demonstrated statistical significance ($p < 0.05$) in the bivariate analysis, the unadjusted prevalence ratio (PR) was calculated to determine the initial strength of the association.

Finally, to identify independent clinicopathological predictors of high c-MET expression, variables demonstrating statistical significance in the chi-square test were incorporated into a multivariate analysis. A Poisson regression model with a robust variance estimator was utilized for this purpose, allowing for the direct calculation of the adjusted prevalence ratios (aPR) alongside their 95% confidence intervals (CI). A two-tailed p -value of less than 0.05 was considered statistically significant.

Ethical Approval

This study has received approval from the Research Ethics Committee of the Faculty of Medicine, Udayana University / Ngoerah Hospital, Denpasar, with ethical clearance number 0052/UN14.2.2.VII.14/LT/2026 and has been granted permission by Ngoerah Hospital, Denpasar, with letter number DP.04.03/D.XVII.2.2.2/5547/2026.

RESULTS

Characteristics of the Research Samples

Based on data from 40 research samples collected at Ngoerah Hospital, Denpasar, the distribution of clinicopathological characteristics is presented in Table 1. The

sample distribution by sex demonstrated a male predominance with 21 cases (52.5%), while females accounted for 19 cases (47.5%). Regarding age, the ≥ 60 years cohort represented a higher proportion with 24 cases (60.0%) compared to the < 60 years cohort with 16 cases (40.0%).

In terms of pathological parameters, the majority of the samples exhibited a non-mucinous adenocarcinoma histological subtype, comprising 31 cases (77.5%). The degree of differentiation was similarly dominated by a low degree of differentiation in 31 cases (77.5%). Regarding tumor extension, most samples demonstrated a depth of invasion of pT3-4, observed in 24 cases (60.0%). Additionally, a positive nodal status (lymph node metastasis) was identified in 25 cases (62.5%).

Table 1. Distribution of research sample characteristics

Sample characteristics		Frequency	Percentage
Sex	Male	21	52.5%
	Female	19	47.5%
Age	< 60 years	16	40.0%
	≥ 60 years	24	60.0%
Histological subtype	Non-mucinous adenocarcinoma	31	77.5%
	Mucinous adenocarcinoma	9	22.5%
Degree of differentiation	Low	31	77.5%
	High	9	22.5%
Depth of invasion	T1-T2	16	40.0%
	T3-T4	24	60.0%
Nodal status	Negative	15	37.5%
	Positive	25	62.5%

Furthermore, the evaluation of c-MET immunohistochemical (IHC) staining was conducted using a semi-quantitative approach known as the Histo-Score (H-Score), which incorporates the assessment of both staining intensity and the percentage of stained cells. The staining intensity was assigned an index score of 0, 1+, 2+, or 3+, corresponding to negative, weak, moderate, and strong intensities, respectively (Figure 1).

A Shapiro-Wilk normality test for H-Score revealed a non-normal data distribution ($p < 0.001$); consequently, the median H-Score of 150 was established as the cut-off value. As outlined in Table 2, samples with an H-Score below 150 were classified as exhibiting low c-MET expression, whereas those with an H-Score greater than or equal to 150 were categorized as having high c-MET expression. Based on these criteria, high c-MET expression was observed in 23 samples (57.5%), and low c-MET expression was identified in 17 samples (42.5%).

Table 2. Frequency Distribution of c-MET Expression H-Scores

H-Score	Number
Low	17 (42.5%)
High	23 (57.5%)

Association Between c-MET Expression and Histological Subtypes of CRA

The association between c-MET expression and histological subtypes in this study was analyzed using the chi-square test, yielding the results presented in Table 3. Based on this chi-square

analysis, it can be concluded that there is no statistically significant association between c-MET expression and the histological subtypes of colorectal adenocarcinoma (CRA), with a p -value of 0.893 ($p > 0.05$).

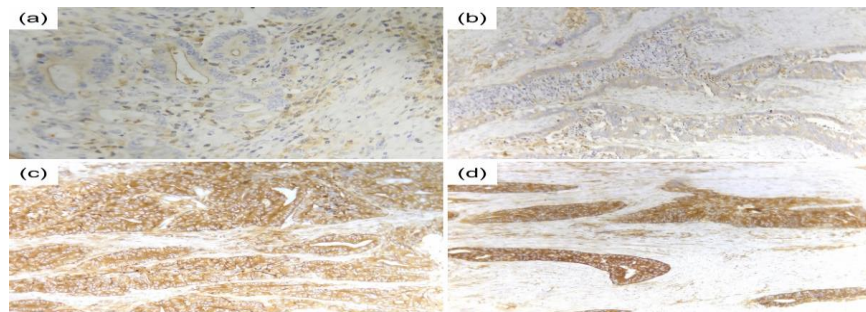


Figure 1. c-MET IHC expression in CRC at 100x magnification. (a) Negative staining (0), (b) Weak intensity (1+), (c) Moderate intensity (2+), and (d) Strong intensity (3+)

Association Between c-MET Expression and the Depth of Invasion in CRA

The association between c-MET expression and the depth of invasion of colorectal adenocarcinoma (CRA) in this study was evaluated using the chi-square test, with the results detailed in Table 3. Based on this chi-square analysis, it can be concluded that there is a statistically significant association between c-MET expression and the depth of invasion in colorectal adenocarcinoma, demonstrating a p -value of 0.006 ($p < 0.05$).

Subsequently, further statistical analysis was conducted to determine the strength of the association by calculating the prevalence ratio (PR) between c-MET expression and the depth of invasion of CRA in this study, as presented in Table 4. Subjects with high c-MET expression were found to have a 2.400-fold higher risk of experiencing deeper tumor invasion (pT3-4) compared to subjects with low c-MET expression.

Association Between c-MET Expression and the Degree of Differentiation in CRA

The association between c-MET expression and the degree of differentiation in this study was analyzed using the chi-square test, yielding the results presented in Table 3. Based on this chi-square analysis, it can be concluded that there is a statistically significant association between c-MET expression and the degree of differentiation in colorectal adenocarcinoma (CRA), demonstrating a p -value of 0.003 ($p < 0.05$).

Subsequently, further statistical analysis was conducted to determine the strength of this association by calculating the prevalence ratio (PR) between c-MET expression and the degree of differentiation in CRA, with the findings presented in Table 4. Subjects exhibiting high c-MET expression were found to have a 2.214-fold greater risk of developing CRA with a high degree of differentiation compared to subjects with low c-MET expression.

Table 3. Association between c-MET expression and pathological features

Pathological features	Low c-MET (n=17)	High c-MET (n=23)	p*
Histological subtype			0.893
Mucinous	4 (23.5%)	5 (21.7%)	
Non-mucinous	13 (76.5%)	18 (78.3%)	
Degree of differentiation			0.003
Low	17 (100%)	14 (60.9%)	
High	0 (0.0%)	9 (39.1%)	
Depth of invasion			0.006
pT1-2	11 (64.7%)	5 (21.7%)	
pT3-4	6 (35.3%)	18 (78.3%)	
*Nodal status			0.002
Negative	11 (64.7%)	4 (17.4%)	
Positive	6 (35.3%)	19 (82.6%)	

*Statistically significant if $p < 0.05$

Association Between c-MET Expression and Nodal Status in CRA

The association between c-MET expression and the nodal status of colorectal adenocarcinoma (CRA) in this study was evaluated using the chi-square test, with the results presented in

Table 3. Based on this chi-square analysis, it can be concluded that a statistically significant association exists between c-MET expression and nodal status in CRA, demonstrating a p -value of 0.002 ($p < 0.05$).

Subsequently, further statistical analysis was conducted to determine the strength of this association by calculating the prevalence ratio (PR) between c-MET expression and nodal status in this study, with the findings detailed in Table 4. Subjects

exhibiting high c-MET expression were found to have a 2.850-fold higher risk of experiencing nodal metastasis (positive nodal state) compared to subjects with low c-MET expression.

Table 4. Prevalence ratio between high c-MET expression and pathological features

Pathological features	Prevalence ratio (PR)	95% Confidence interval (CI)
Degree of differentiation	2.214	(1.502 - 3.264)
Depth of invasion	2.400	(1.119 - 5.145)
Nodal status	2.850	(1.197 - 6.787)
Number (N)	40	

Multivariate Poisson Regression Analysis with Robust Standard Error

A multivariate Poisson regression with robust standard error was conducted to evaluate independent predictors of high c-MET expression (Table 5), excluding histological subtype due to prior bivariate non-significance ($p=0.274$). Positive nodal status emerged as the strongest independent factor, increasing the probability of high c-MET expression by 2.31-fold (1/0.433) compared to negative nodal status (Adjusted prevalence ratio (aPR)=0.433; 95% CI: 0.195–

0.961). Similarly, a high degree of differentiation increased this probability by 1.62-fold (1/0.618) compared to low differentiation (aPR=0.618; 95% CI: 0.427–0.893). Conversely, depth of invasion was not a statistically significant independent factor; although pT3-4 tumors demonstrated a 1.54-fold greater tendency for high c-MET expression than pT1-2, the confidence interval encompassed 1 (aPR=0.649; 95% CI: 0.299–1.408). Ultimately, nodal status and degree of differentiation were independently associated with increased c-MET expression.

Table 5. Multivariate Poisson Regression with robust standard error

Pathological Features	Adjusted prevalence ratio	95% Wald Confidence Interval	p*
Low degree of differentiation	0.618	(0,427 – 0,893)	0.010
pT1-2 depth of invasion	0.649	(0,299 – 1,408)	0.274
Negative nodal Status	0.433	(0,195 – 0,961)	0,040

*Statistically significant if $p < 0.05$

DISCUSSION

Based on the research findings of 40 colorectal adenocarcinoma (CRA) samples at Ngoerah Hospital, Denpasar, the majority of subjects were male (52.5%) and aged ≥ 60 years (60.0%). These findings align with literature stating that biological aging, associated with immune senescence and the prolonged accumulation of genetic and epigenetic alterations, is a primary internal factor in CRA carcinogenesis.^{7,24,25} The male predominance is frequently attributed to higher exposure to carcinogenic lifestyle factors, while estrogen exerts a protective effect on female colonic mucosa via estrogen receptor beta.^{6,7} Pathologically, most samples exhibited a high-differentiation, non-mucinous adenocarcinoma subtype (77.5%) and presented with an aggressive disease profile: pT3-pT4 invasion depth (60.0%) and positive nodal status (62.5%). This advanced presentation reflects low rates of early screening and synchronizes with high lymph node metastasis [26]. Utilizing a median H-Score cut-off of 150, 57.5% of samples exhibited high c-MET expression, surpassing previous studies (40-50%).²⁷⁻²⁹ This c-

MET overexpression indicates pathological activation via transcriptional dysregulation, with or without gene amplification.^{30,31}

Statistical analysis revealed no significant association between c-MET expression and CRA histological subtypes ($p=0.893$), indicating that oncogenic c-MET activation occurs across morphological variants without phenotypic preference. As a receptor tyrosine kinase, c-MET drives cellular proliferation, apoptosis evasion, and EMT^{7,30}—activities determining tumor aggressiveness rather than cellular differentiation traits like mucin synthesis.^{1,9,10,32-35} While specific subtypes harbor distinct genetic aberrations (e.g., BRAF/MSI), c-MET dysregulation transcends WHO morphological criteria.⁷ Furthermore, the dominance of the non-mucinous subtype (77.5%), mirroring global epidemiological trends ($>95\%$), created an imbalance that contributed to this non-significant result.⁸ Conversely, a significant association was found with the degree of differentiation ($p=0.003$; PR=2.214, 95% CI: 1.502-3.264), with all highly differentiated samples demonstrating

high c-MET levels. This links excessive c-MET activation to the loss of glandular architecture via the EMT pathway. Activation of the canonical c-MET/MAPK cascade induces EMT transcription factors (cyclin D1, MYC, TWIST, SNAIL, ZEB, SLUG), leading to a "cadherin switch".^{6,7,24,25,36,37} The resulting repression of E-cadherin and upregulation of vimentin/N-cadherin causes cells to detach, lose polarity, and form the solid structures characteristic of a high degree of differentiation.^{7,38}

In bivariate analysis, high c-MET expression significantly correlated with depth of invasion (pT3-pT4) ($p=0.006$ / $p<0.005$; PR=2.400, 95% CI: 1.119-5.145). However, the significance of invasion depth was lost when tested in a multivariate model alongside the degree of differentiation and nodal status (95% Wald CI including the value of 1). This indicates that the depth of invasion is not an independent factor of c-MET expression in CRA. The loss of statistical significance is likely due to the confounding effects of nodal status and degree of differentiation; pathobiologically, tumors with deeper invasion (pT3-pT4) possess an inherently higher propensity for lymphogenic spread (positive nodal status). Therefore, the bivariate association between depth of invasion and c-MET is essentially represented by nodal status as the more independent primary driver. Mechanistically, tumor progression into deeper wall layers necessitates aggressive stromal destruction, facilitated by the c-MET-induced secretion of Matrix Metalloproteinases (MMP-2, MMP-9) and urokinase plasminogen activator (uPA).^{6,32} Concurrently, c-MET activation triggers actin cytoskeleton reorganization and focal adhesion kinase (FAK) phosphorylation, transforming neoplastic cells into motile clones.^{30,36} Following EMT-driven detachment via the PI3K/Akt and MAPK pathways, these highly migratory cells exploit chemotactic gradients to breach thin-walled local lymphatic vessels.^{6,7,30,32,38} This lymphogenic dissemination—the stronger independent driver—is further accelerated by c-MET stimulating lymphangiogenesis factors (VEGF-C and VEGF-D), which bind to peritumoral VEGFR-3 to provide transportation routes to regional lymph nodes.⁶

This study possesses several methodological strengths that reinforce its clinical findings, including the use of a highly sensitive and specific intracellular c-MET antibody, the objective H-Score system for evaluating staining intensity and proportion, and a statistically robust median cut-off derived from normality testing. Collectively, these successfully confirm the biological role of the HGF/c-MET signaling pathway in driving tumor aggressiveness. However, several limitations exist. The relatively small sample size ($n=40$) obtained via consecutive sampling may restrict the broader generalizability of the findings. Additionally, reliance on a single paraffin tissue block may not capture the spatial heterogeneity of c-MET expression, particularly at the invasive front where EMT frequently occurs. The study is also limited to protein-level evaluation via IHC; without data on gene amplification (FISH/SISH) or mRNA levels (RNAscope), other dimensions of c-MET dysregulation cannot be definitively identified. Finally, the cross-sectional design precludes the evaluation of long-term clinical outcomes, such as overall or progression-free survival.

CONCLUSIONS AND RECOMMENDATIONS

Based on the findings of this study, several conclusions can be drawn regarding colorectal adenocarcinoma (CRA) at Ngoerah Hospital, Denpasar. First, there is no association between c-MET expression and the histological subtypes of CRA. However, a significant association exists between c-MET expression and the degree of differentiation; specifically, subjects with high c-MET expression have a 2.214-fold higher risk of developing CRA with a high degree of differentiation compared to those with low expression. Furthermore, an association was found between c-MET expression and the depth of invasion, indicating that subjects with high c-MET expression possess a 2.400-fold greater risk of experiencing deeper tumor invasion (pT3-4) relative to subjects with low c-MET expression. Finally, there is an association between c-MET expression and nodal status, wherein subjects exhibiting high c-MET expression have a 2.850-fold increased risk of experiencing nodal metastasis (a positive nodal status) compared to subjects with low c-MET expression.

In light of these findings, several recommendations are proposed. The immunohistochemical (IHC) evaluation of c-MET may be considered as an additional prognostic parameter to assess the risk of tumor aggressiveness and metastasis in patients with CRA. Moreover, to address the challenge of intratumoral heterogeneity, future research is advised to utilize a hybrid scoring system or to integrate protein-level assessment (IHC) with mRNA evaluation via RNAscope to achieve improved stratification accuracy. Ultimately, subsequent studies with larger sample sizes are highly recommended to definitively validate the role of c-MET as a predictive biomarker for assessing responses to targeted therapies in the future.

Conflict of Interest

There is no conflict of interest in this study.

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None.

Author Contributions

All authors have made equal contributions to the writing of this research, from the development of the conceptual framework, data collection, data analysis, to the interpretation of the research results.

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