Pakistan J. Zool., pp 1-12, 2025. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20250520135756

c-MET as a Prognostic Biomarker for Colon Cancer Progression, Metastasis, and Targeted Therapy

Fatima Khalid[1]*, Zahoor Qadir Samra[1] and Muhammad Imtiaz Shafiq[2]

[1]*School of Biochemistry and Biotechnology, University of the Punjab, Quaid-i-Azam Campus, Lahore*
[2]*School of Chemistry, University of the Punjab, Quaid-i-Azam Campus, Lahore*

ABSTRACT

Colorectal cancer (CRC) is among the leading causes of cancer-related mortality globally, with metastasis as a key driver of poor outcomes. In Pakistan, colon cancer is the fourth most prevalent cancer, with an increased occurrence rate in young people. The c-MET/HGF axis plays a significant role in tumor budding, proliferation, invasion, and metastasis. This study investigates c-MET expression in human colon cancer and methylnitrosourea (MNU)-induced mouse colon cancer models to establish its clinical significance and therapeutic potential. Using quantitative real-time PCR (qPCR) and immunohistochemistry (IHC), c-MET expression was found to correlate positively with tumor stage and metastasis in both human and mouse tissue samples, with significant p-values ($P < 0.05$). Advanced analysis, including immunofluorescence, confirmed the presence of c-MET receptor, highlighting its diagnostic and therapeutic potential. Importantly, a strong correlation between c-MET and HGF expression (human: Pearson correlation = 0.707, $P < 0.0005$) underscores the role of this axis in CRC pathogenesis. These findings suggest that targeting c-MET may offer a promising strategy for early diagnosis and effective treatment of colon cancer, addressing metastasis, and overcoming current therapeutic limitations.

Article Information
Received 20 May 2025
Revised 25 July 2025
Accepted 05 August 2025
Available online 15 December 2025 (early access)

Authors' Contribution
FK, ZQS: Conceptualization and design of study. FK, MIS:Analysis and interpretation of data. FK: Drafting of the article, acquisition of data. FK, ZQS, MIS: Revising content, final approval of version.
All authors read and agreed to the published version of the manuscript.

Key words
c-MET, Colon cancer, Targeted therapy, Prognostic biomarker, Immunohistochemistry, qPCR

INTRODUCTION

Colorectal cancer (CRC) is considered to be the third major cause of death linked to cancer across the globe (Danese and Montagnana, 2017), accounting for approximately 10% of all cancer cases and standing as the second leading cause of cancer-related deaths (World Health Organization, 2023 report). The incidence of CRC and its mortality rate in cancer patients is increasing at an alarming rate, particularly in low and middle-income countries. In addition, the worldwide burden of CRC is expected to increase by more than 60%, with approximately 2.25 million new cases and 1.2 million deaths, according to predictions for 2030 (Sung *et al*., 2021; Seigel *et al*., 2024; Bray *et al*., 2024). The death rate linked to CRC is mainly linked to its metastasis, primarily in the liver, lung, brain, and lymph nodes, and this accounts for one of the momentous causes of death in patients suffering from CRC. According to the prior literature studies, around 25% of the patients exhibit colorectal metastasis at the time of initial treatment, which advances to nearly 50% during the later stages of cancer and correlates with our current study (Nakayama *et al*., 2013; Cleveland Clinic, 2022). However, the currently available gold standard therapies for treating CRC and its related metastasis include surgery, radiotherapy, and chemotherapy, which still seem less efficient in targeting this silent killer, which starts to show its signs when much damage has already been done (Cyr *et al*., 2023).

In Pakistan, colon cancer is the fourth most prevalent cancer, with survival rates varying between 14% and 90%, depending on the stage at diagnosis and early detection (Ahmed *et al*., 2024). Recent studies have highlighted a concerning rise in colon cancer cases among younger individuals in the country, a trend attributed to factors such as changing dietary habits, sedentary lifestyles, and genetic predispositions (Rani, 2024). This shift underscores the

* Corresponding author: fatimakhalid6688@gmail.com
0030-9923/2025/0001-0001 $ 9.00/0

Abbreviations
H and E, hematoxylin and eosin; OCT, optimal cutting temperature; IHC, immunohistochemistry; DAB, diaminobenzidine; FITC, fluorescein isothiocyanate.

urgent need for increased awareness, early screening, and preventive measures to address the growing burden of colon cancer in Pakistan. Moreover, the absence of population-specific diagnostic markers and therapeutic targets limits the effectiveness of existing approaches (National Cancer Institute, 2024). The receptor tyrosine kinase c-Met has emerged as a promising biomarker and therapeutic target in colon cancer. Overexpression of c-Met is associated with tumor growth, invasion, metastasis and aggressiveness and has been linked to poor prognosis in CRC patients (Liu, 2015).

Hepatocyte growth factor (HGF) and its linked c-MET receptor have tyrosine kinase activity and are significantly involved in epithelial to mesenchymal transition (EMT), associated with many biological functions leading towards growth, differentiation, and migration (Sierra and Tsao, 2011; Bradley *et al*., 2016; Jin *et al*., 2024; Uchikawa *et al*., 2021). Under normal physiological conditions, the c-MET/HGF axis interaction leads toward cellular proliferation, migration, and angiogenesis and also aids in the wound healing process. On the other hand, in many cancers, the c-MET/HGF pathway is viewed as an important contributor to tumor invasion and proliferation due to the alternation of genes and overexpression of the c-MET receptor, which eventually leads toward metastasis (Sun *et al*., 2013; Barzaman *et al*., 2022; Lee *et al*., 2021; Recondo *et al*., 2020). Despite increasing meta-analysis for the c-MET/HGF pathway, its exact role in CRC and concurrent metastasis is undermined.

Our study aims to investigate the expression of c-Met in human colon cancer samples from Pakistani patients and in a mouse model induced with N-methyl-N-nitrosourea (MNU). By identifying c-Met expression patterns, we hope to establish its utility as a diagnostic marker and explore its potential as a target for drug delivery. This approach could lead to improved early detection and the development of more effective, targeted therapies, addressing the limitations of current methods and providing a population-specific solution to enhance colon cancer outcomes in Pakistan.

MATERIAL AND METHODS

Human cohort

With the ethical approval from Institutional Ethics Review Board, University of Punjab, Lahore (Ref No. 285/SBB). The cancer tissue sections were cut vertically into two parts; one was preserved in 10 % formalin, and the other was immediately stored in liquid nitrogen to snap-freeze the sample and later stored at -80 °C till further usage. The human cohort comprised 72 samples, 60 colon cancer patients (38 males and 22 females), and 12 healthy individuals. Most of the colon cancer patients (27) were of stage 1 and 2 cancer patients, while 18 were of stage 3 and 15 patients were of stage 4. The average age was 56 (range 35-78) years. A total of 31 patients underwent tumor resection surgery for the primary tumor, and 29 patients went for the removal of both primary and resectable metastatic sections. All the patients who underwent surgical resection were further analyzed by post-operative examination. In addition, adjuvant therapies, such as radiotherapy and chemotherapy, were also given to the prescribed patients.

Mouse colon cancer model

Mice were divided into two groups; one was dissected after 6 months and the other after 12 months. An equal number of males and female mice were kept in both groups. Initially, 8 mice (4 male and 4 females) were kept in group 1, while 14 mice (7 males and 7 females) were kept in group 2. During the process of cancer induction, 2 mice died from group 1 and 4 mice died from group 2 leaving a total of 6 mice in group 1 and 10 mice in group 2 (Lodhi *et al*., 2021). Four healthy albino mice were used in the study. No mortality was reported in control mice group. Colon cancer was induced in mice through a combination of N-methyl-N-nitrosourea (MNU) and high salt (NaCl) concentration. Group 1 mice were dissected after 6 months, while Group 2 mice were dissected after 12 months of cancer induction as shown Table I. After the mice dissection, colon tumor tissues were collected from both groups and were cut into two parts vertically; one was preserved in 10 % formalin, and the other was immediately stored in liquid nitrogen to snap-freeze the sample and later stored at -80 °C till further usage (Lodhi *et al*., 2021).

Table I. Histopathological profile of mice colon cancer induced tissues after interval of 6 months and after 12 months.

Findings	After 6 months	After 12 months
No of total animals	8	14
Dead during infection	2	4
Percentage of cancer incidence	42%	80%
Uneven surface and hemorrhage	4	10
Presence of inflammatory cells	7	7
Mucosal and submucosal abscess	8	9
GIST	3	4
Signet ring cell carcinoma	0	2
Polypoid adenocarcinoma	2	3
Dysplasia	1	5
Adenocarcinoma	3	7
Lymphoma	0	1

Table II. Primer sequences for human and mouse genes.

Gene	Upstream/ Downstream primer sequence	Base pair length (bp)	Temperature (°C)	GC content (%)
Human primers				
c-MET	5'-TGTCTTGGGGGTTCAGATAA-3'	120 bp	59	45
	5'-ACAGATTAGCAGGGCTTGTG-3'		60	50
HGF	5'-TGGTTCTGATTGCCCCTGAT-3'	162 bp	60	50
	5'-TCTACCCTTGCTTGCTGACA-3		60	50
TBP	5'-CCTACTCTGCTGTGTTTCGC-3	132 bp	60	50
	5'-GGGCAGGGTAAACTTAGGGT-3'		60	55
Mouse primers				
c-MET	5'-CCCCACACAGTAGCCAAGAT-3'	195 bp	60	55
	5'-GAAGAGGACGCTCACCAGTC-3'		60	60
HGF	5'-TCCCTCGTCAGCTTGTCTTT-3'	179 bp	60	50
	5'-CCTCCAAATGTCCATGCTCT-3		59	50
TBP	5'- GCAGCCTCAGTACAGCAATC-3'	98 bp	60	55
	5'-GGTGCAGTGGTCAGAGTTTG-3'		60	55

Real-time PCR for gene expression analysis

RNA was isolated from the frozen tissue samples of both humans and mice employing the TRIzol reagent followed by DNase treatment using the RNase-Free DNase Set (Qiagen, catalogue #79254) to eliminate genomic DNA. 1 µg of the isolated RNA was then reversely transcribed into cDNA under sterile conditions using the oligo dT primers by RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific catalogue #4368814), according to the manufacturer's protocol.

The gene specific expression primers were used for c-Met expression studies in humans and mice, given in Table II. TATA binding protein (TBP) was used as an endogenous reference control due to its consistent expression behavior in normal and cancer tissues. The 10µl reaction consisted of 5µl (1 X) SYBR dye (Thermo Fisher Scientific, catalogue # K0221), 0.35 µl (0.3 µM) upstream and downstream primer, 1µl (10ng) cDNA template, and 3.3 µl RNAase free water. The reaction was carried out as: initial denaturation at 95°C for 10 min followed by 40 cycles of PCR reaction: 95 °C denaturation for 30 sec, annealing and extension at 55°C for 45 sec. After the reaction, gene expression was quantified following Livak method (Livak and Schmittgen, 2001). Every reaction was carried out in triplicate to ensure consistent results.

Characterization of c-MET receptor

Immunohistochemical analysis

Histological study including H and E staining was performed on formalin fixed paraffin embedded (FFPE) tissue section of both human and mice colon cancer. For H and E staining, tissue sections were first deparaffinized using xylene and rehydrated using descending concentration of alcohol (i.e., 95%, 90% and 70%). After rehydration, tissue sections were stained with hemolysin stain following washing the slides with weak acid and mild alkaline solution alternatively for better differentiation and bluing effect. Later, tissue sections were counterstained with eosin stain to visualize cellular contents of the colon cancer tissue sections under microscope.

Immunohistochemistry (IHC) was also performed for tissue sections of both humans and mice, which showed varied expression in quantitative real-time PCR (qPCR) analysis according to the defined protocol. Briefly, 3µm of FFPE tissue sections were cut with the help of a microtome, placed on a glass slide, and oven-dried overnight.

After deparaffinizing tissue sections, slides were exposed to 3% H_2O_2 for 10 min to hinder the endogenous activity. In order to recover the receptor activity, slides were placed in Tris Cl buffer (pH 9.0) for 10 min at 100°C. Tissue sections were incubated with primary anti-c-MET antibody (1:100) for 1 h at room temperature, followed by goat anti-mouse HRP conjugated secondary antibody (1:2000) for 1 h at room temperature (Lodhi *et al*., 2021). The reaction showed results after incubation with 1% diaminobenzidine (DAB) substrate with 3% H_2O_2 and images were captured using florescent microscope, Olympus BX51.

Post-staining, slides were digitized and analyzed with Aperio ImageScope. Analysis including quantifying expression positivity, with semi-quantitative scoring (i.e., 0

to +4 scale) applied in collaboration with a histopathologist.

Immunofluorescence

Frozen human and mouse colon cancer tissue sections were dehydrated with different concentrations of sucrose (i.e., 5%, 10%, 20%, and 30%). These sections were embedded in OCT and stored at -20 ºC. Tissue sections of around 8 µm were precisely cut on a cryostat and placed on the albumin-coated slides (Lodhi *et al*., 2021). Sections were placed in the humified chamber and blocked with 5% skimmed milk solution for 1 h. The slides were washed with TBS solution and incubated with primary anti-c-MET antibody (1:100) for 1 h. After primary antibody incubation, slides were washed and incubated with fluorescent-labeled secondary antibodies (FITC conjugated antibody and Texas Red labeled antibody) in 1:2000 dilution for 1 h at 37ºC. Slides were observed under the fluorescent microscope's blue and green excitation filters (Lodhi *et al*., 2022).

Statistical analysis

The SPSS (version 25.0) software (SPSS Inc., Chicago, IL, USA) was used for statistical analysis. A one-way ANOVA test was employed to study multiple groups under consideration, and an unpaired Student t-test, also known as an independent t-test, was considered for studying two groups. For analysis, an P value less than 0.05 was considered statistically significant. Pearson correlation analysis was used to study the correlation between c-MET and HGF, and a P value less than 0.01 was taken as statistically significant.

RESULTS

Clinicopathological features of colon cancer subjects

Tables III and IV show the clinicopathological features of humans and mice. In human cohort, the distribution of TNM stage (Tumor, Node, and Metastasis staging system) showed that 7 patients presented with stage I, 20 with stage II, 18 with stage III (metastatic), and 15 with stage IV cancer with a severe degree of metastasis.

In mouse cohort, the distribution of TNM stage in group 1 showed that 4 mice developed stage I cancer while 2 mice had stage II cancer. In group 2, 3 mice progressed to stage II cancer, 3 into stage III cancer, and 4 mice developed stage IV metastatic colon cancer in total.

Expression and prognostic significance of c-MET in human and mouse colon cancer

Human colon cancer

To validate the c-MET expression from clinical specimens, tissue samples were taken from primary and metastatic colon cancer patients. In metastatic colon cancer samples, cancer had spread mainly to the liver (12 samples), lung (8 samples), brain (5 samples), lymph nodes (9 samples), and peritoneum (6 samples). Real-time

Table III. The relationship of c-MET expression with clinicopathological features in human colon cancer.

Clinicopathologic features	n	F	P value
Gender			
Male	38	0.422	0.519
Female	22		
Age			
> 60	37	2.273	0.137
≤ 60	23		
Tissue differentiation grade			
Low	16	2.404	0.131
Medium	29		
High	15		
Stage			
I and II	27	4.634	0.014
III	18		
IV	15		
Metastasis			
With	46	5.810	0.019
Without	14		

Table IV. The relationship of c-MET expression with clinicopathological features in induced mouse colon cancer for 6 months and 12 moths.

			After 6 months	After 12 months
Clinicopathologic features	n	F	P-value	P-value
Gender				
Male	4	0.496	0.520	0.895
Female	2			
Tissue differentiation grade				
Low	3	3.718	0.126	0.104
Medium	3			
High	0			
Stage				
I and II	4	1.395	0.303	0.040
III	2			
IV	0			
Metastasis				
With	3	15.041	0.018	0.015
Without	3			

quantitative reverse transcription polymerase chain reaction (qPCR) showed that c-MET expression significantly increased in metastatic colon cancer samples with a fold change (FC) = 5.31 ± 0.87 beside primary colon cancer tissue sections, FC = 2.73 ± 0.84. While the fold change expression of HGF in the metastatic colon cancer tissue sections increased to 5.09 ± 0.76 as compared to primary colon cancer tissue sections (FC = 2.58 ± 0.93).

To elucidate the prognostic significance of c-MET in both primary and metastatic colon cancer tissues, clinicopathological correlation of c-MET at mRNA was studied in 60 colon cancer tissue samples. Univariate analysis showed that the expression level of mRNA for c-MET positively correlated with increasing tumor stage and rate of metastasis. Multivariate analysis, on the other hand, showed that age, gender, and tumor-differentiated grade are independent prognostic factors. These findings indicated that c-MET is activated in colon cancer; its expression increases drastically with increasing stage of cancer and is an important contributor in colon cancer metastasis, mainly to the liver, lymph nodes, lungs, and peritoneum. This suggests that c-MET signaling and its targeted therapy could help suppress tumor invasion and its subsequent metastasis.

Mouse colon cancer

c-MET expression was also validated in the methyl nitroso urea-induced colon cancer mouse model, where samples were taken from both primary and metastatic colons. In metastatic colon cancer samples, cancer had spread mainly to the liver (4 samples), lung (1 sample), lymph nodes (3 samples) and peritoneum (2 samples). Real-time quantitative reverse transcription polymerase chain reaction (qPCR) showed that c-MET expression significantly increased in metastatic colon cancer samples (FC = 5.36 ± 0.27) as compared to primary colon cancer tissue sections (FC= 2.16 ± 1.03). While the fold change expression of HGF in the metastatic colon cancer tissue sections increased to 5.76 ± 0.64 as compared to primary colon cancer tissue sections (FC = 2.31 ± 1.24).

The prognostic significance of c-MET was also elucidated in both primary and metastatic colon cancer tissues; the clinicopathological correlation of c-MET at mRNA was studied in 16 colon cancer-induced mouse tissue samples. Univariate analysis showed that the expression level of mRNA for c-MET positively correlated with increasing tumor stage and rate of metastasis. Univariate and multivariate analyses of mouse colon cancer tissue sections showed similar results aligned with human analyses, suggesting that methylnitrosourea-induced colon cancer animal models can be used to study the c-MET targeted therapy for tumor invasion and metastasis.

Correlation between c-MET and HGF in colon cancer tissue sections

Pearson correlation analysis assessed the correlation between c-MET and HGF in human and mouse colon cancer tissue sections. Both the genes had a positive correlation for human cancer samples ($r = 0.707$, $p < 0.0005$), as shown in Table V. The mouse cohort also showed a positive correlation between c-MET and HGF. In group 1, when the tumor was in the primary stage, both genes were positively correlated with a value of 0.344 but were not statistically significant. On the other hand, in Group 2, as cancer progressed, the correlation between c-MET and HGF became strong, with a correlation coefficient of 0.777 and $p = 0.008$, as shown in Table V.

Table V. Correlation between c-MET and HGF in human colon cancer tissue samples.

No of samples	Pearson correlation	Significance
Human colon cancer tissue samples		
60	0.707	> 0.0005
Induced mouse colon cancer tissue samples		
Group 1 (6 samples)	0.344	0.505
Group 2 (10 samples)	0.777	0.008

Expression of c-MET in colon cancer tissue using IHC

To validate the expression of c-MET receptor protein in the tissue samples of both humans and mice, the tissue samples were subjected to both H and E staining and IHC. c-MET receptor protein was found in all the colon cancer tissue samples with varied expression levels. Figure 1 a-e shows H and E staining while section f-j shows IHC of human normal and colon cancer tissue sections. For human samples, 90% showed +2 or higher intensity expression level of c-MET receptor, as shown in Figure 1 and Table VI. Similar results were found for mouse colon cancer tissue samples, where 81.25% of tissue samples showed +2 or higher-level intensity expression for the c-MET receptor, respectively, as shown in Figure 2 and Table VI. Figure 2 section a-e shows H and E staining while section f-j shows IHC of mouse normal and colon cancer tissue sections. On the other hand, normal colon tissue sections from both humans and mice were devoid of c-MET receptor expression. The results suggest that the c-MET receptor could be a biomarker to detect colon cancer progression.

Human and mouse colon cancer tissue sections were processed by immunofluorescence to study the tissue binding ability. The binding of FITC-conjugated secondary

anti-mouse antibodies to the highly expressed c-MET receptor tissue sections, priorly conjugated with anti-c-MET antibodies, gave green fluorescence for both human and mouse colon cancer tissue sections. Red fluorescence was observed when Texas Red labeled anti-mouse secondary antibodies were used for tissue sections priorly conjugated with anti-c-MET antibodies, as discussed earlier. This is illustrated in Figures 3 and 4, representing stage 4 colon cancer for both human and mice.

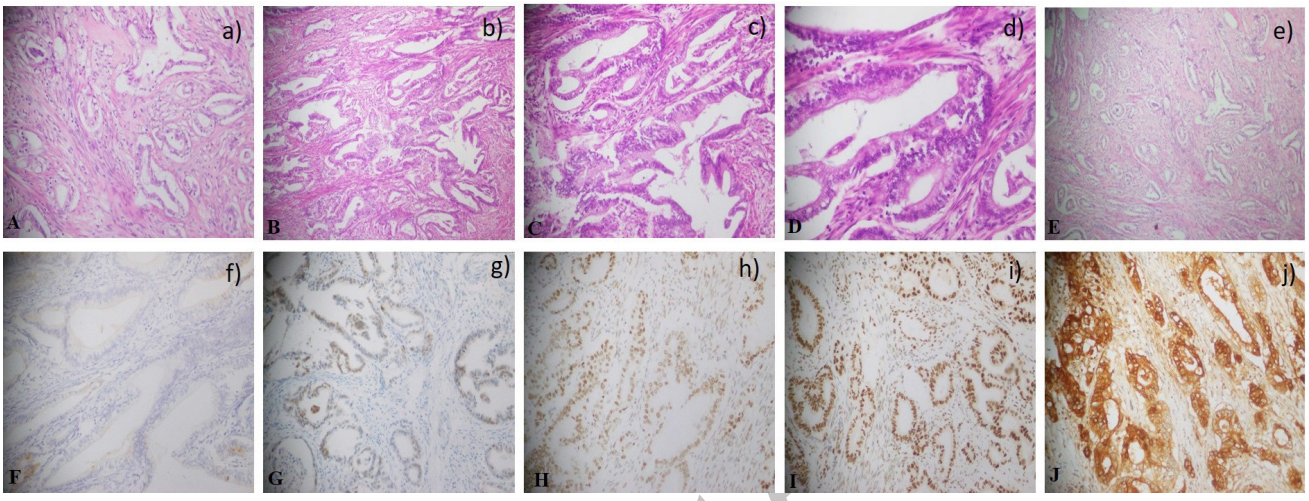

Fig. 1. Expression of c-MET in human colon cancer samples through H and E and IHC staining. Mouse anti - human c-MET monoclonal antibody was used for primary staining in IHC. Florescence Microscopic examination of H and E (A-E) representing (A) normal colon tissue section, (B) stage 1 (C) stage 2, (D) stage 3, (E) stage 4 colon cancer tissue section and IHC (F-J) representing (F) normal colon tissue section, (G) stage 1 (H) stage 2, (I) stage 3, (J) stage 4 colon cancer tissue section staining samples was conducted at 40X, intensity of c-MET expression is numerically presented and their representative images relative to expression intensity are displayed accordingly.

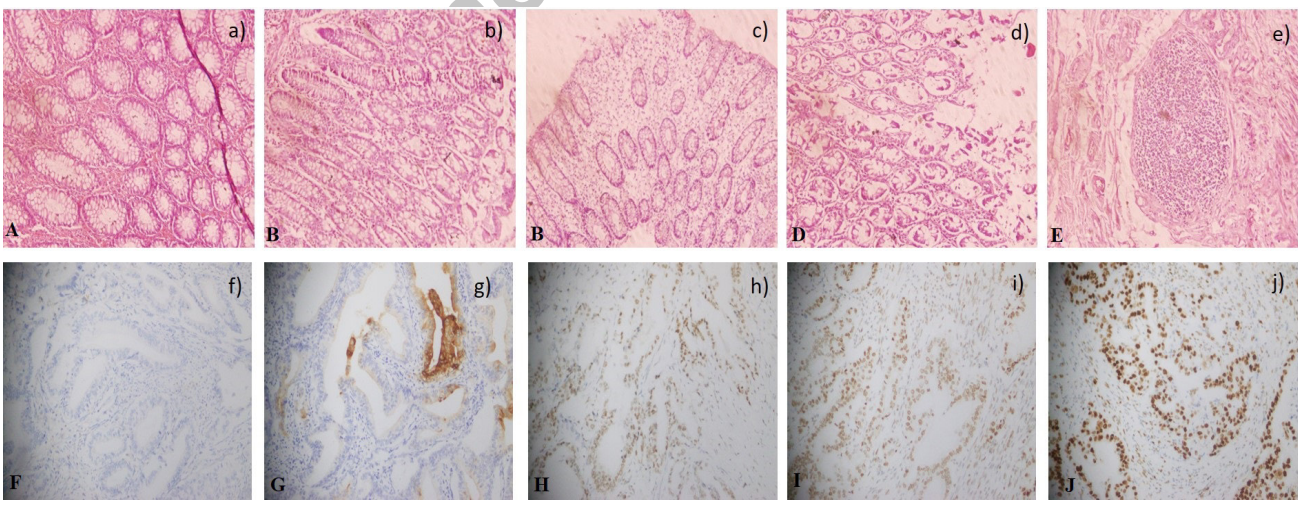

Fig. 2. Expression of c-MET in mouse colon cancer samples through H and E and IHC staining. Mouse anti-human c-MET monoclonal antibody was used for primary staining in IHC. Florescence Microscopic examination of H and E (A-E) representing (A) normal colon tissue section, (B) stage 1 (C) stage 2, (D) stage 3, (E) stage 4 colon cancer tissue section and IHC (F-J) representing (F) normal colon tissue section, (G) stage 1 (H) stage 2, (I) stage 3, (J) stage 4 colon cancer tissue section staining samples was conducted at 40X, intensity of c-MET expression is numerically presented and their representative images relative to expression intensity are displayed accordingly.

Table VI. Expression of c-MET on human colon cancer tissue samples and mouse colon cancer tissue samples using IHC.

	Normal	Stage 1	Stage 2	Stage 3	Stage 4
Human colon cancer tissue sections					
	0	+1	+2	+3	+4
	0	6/60	21/60	18/60	15/60
		10%	35%	30%	25%
Mouse colon cancer tissue sections					
	0	+1	+2	+3	+4
Group 1 (6 months)	0	3/6	2/6	0	0
Group 2 (12 months)	0	0/10	4/10	3/10	4/10
		3/16	6/16	3/16	4/16
		18.75%	37.5%	18.75%	25%

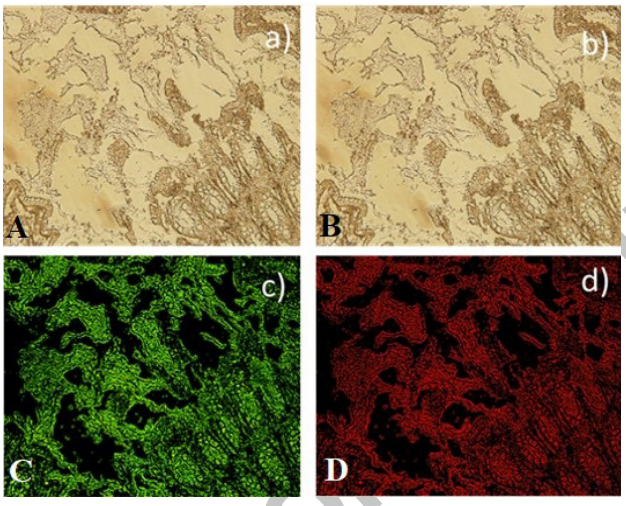

Fig. 3. Immunofluorescence analysis of human colon cancer tissue sections. (A) and (B) represents stage 4 human colon cancer tissue section. (C) represents conjugation between FITC labelled secondary antibody and c-MET antibody by green fluorescence. (D) represents conjugation between Texas Red labelled secondary antibody and c-MET antibody by red fluorescence.

DISCUSSION

CRC cancer is considered the third major contributor to deaths globally, with an increasing number of cases each year (Pennacchietti *et al*., 2003). Identifying certain biomarkers that help in prognosis, diagnosis, and metastatic activity is important. The c-MET gene belonging to the MET family is located on chromosome 7q21 and has 21 exons. HGF and its c-MET receptor form the c-MET/HGF axis, performs many important roles during cell growth and maintenance. c-MET receptor protein in the normal physiology of the cells is considered to be expressed at lower levels, but once activated through various signal transduction pathways, especially involved in cancer cells, its expression exponentially increases (Birchmeier *et al*., 2003; Raghav *et al*., 2016; Tang *et al*., 2015). In addition, the c-MET receptor protein plays a significant role in tumor budding, invasion, and metastasis, which further adds to the aggressive nature of cancer.

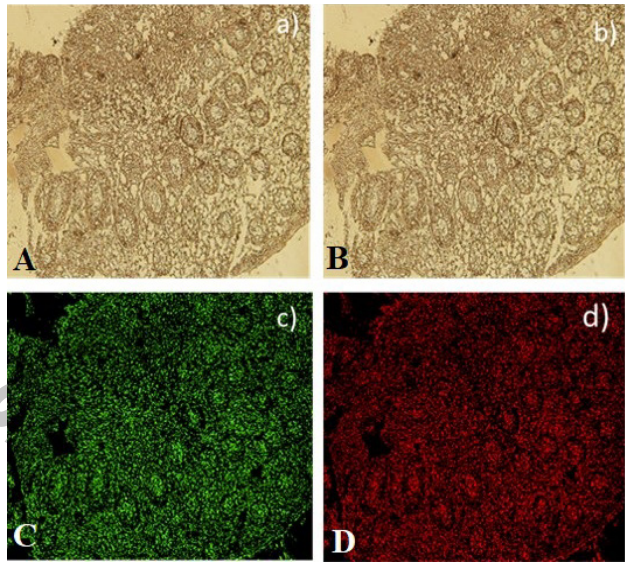

Fig. 4. Immunofluorescence analysis of mouse colon cancer tissue sections. (A) and (B) represents stage 4 mouse colon cancer tissue section. (C) represents conjugation between FITC labelled secondary antibody and c-MET antibody by green fluorescence. (D) represents conjugation between Texas Red labelled secondary antibody and c-MET antibody by red fluorescence.

In the present study, qPCR was employed to evaluate the expression of c-MET and HGF in both human and mouse primary and metastatic colon cancer tissue sections. In addition, IHC was used to reaffirm the presence of c-MET receptor protein on cancerous cells and also in colon cancer tissue samples. The human cohort comprised 72 tissue samples, including 60 colon cancer tissue sections and 12 control samples. Conversely, the mouse cohort consisted of two groups (group 1 and group 2 with varied levels of colon cancer expression) with 4 control mice samples. Results from both the expression analysis procedures showed that c-MET expression had a positive correlation between clinicopathological features of cancer patients, including tumor stage and metastasis (to liver, lungs, brain, and lymph nodes), indicating that c-MET

expression can be considered a substantial prognostic factor in colon cancer metastasis. High c-MET expression was observed in colon cancer tissue from both humans and mice and was directly associated with colon metastasis. Moreover, it was noted that c-MET and HGF had a positive correlation, and together, once overexpressed, it boosted tumor proliferation, invasion, and metastasis to various related organs.

Prior studies have reported that c-MET is linked to primary colon tumor formation, budding, and aggressiveness, but its metastasis to the liver, lung, and brain remains uncertain (Faiella *et al*., 2022). To elucidate the role of c-MET in colon cancer metastasis, we used qPCR analysis, which showed that c-MET expression increased significantly in metastatic colon cancer tissues compared to primary colon cancer tissue sections. It shows that c-MET higher expression in metastatic cancer increases with the increase in tumor stage and its concurrent metastasis to related organs (Kim *et al*., 2013; Cruz *et al*., 2003; Han *et al*., 2024; Wang *et al*., 2020; Wu *et al*., 2018; Terlecka *et al*., 2021). Besides availability of various markers for colon cancer identification including CK20, CDX2 and MUC1; c-MET as a proto-oncogene adds aggressiveness to the developing tumor and aids the process of metastasis. In addition, c-MET being a tyrosine kinase receptor, is a potential candidate for targeted drug delivery applications either through monoclonal antibodies, antibody drug conjugates (ADCs) or certain tyrosine kinase inhibitors.

Immunofluorescence assays further elucidated the presence of c-MET receptor protein on both human and mouse colon cancer tissue sections using FITC and Texas Red labeled secondary antibodies. Our current study showed that targeting c-MET protein receptors could be a promising and exciting therapeutic strategy for patients with colon cancer metastasis.

Recent research-based studies have shown that c-MET inhibitors and other antagonistic agents can be useful in blocking the c-MET signaling cascade, eventually attenuating the growth of cancer (Dong *et al*., 2022). In addition, recently published studies have concluded that MET signaling in lung, liver, and gastric cancer is sensitive to inhibition by using specific tyrosine kinase inhibitors (Nagatsuma *et al*., 2014; Kim *et al*., 2020; Ueno *et al*., 2012; García-Vilas *et al*., 2018a; Ginty *et al*., 2008a). These studies indicate that the c-MET receptor may be an important biomarker in colon cancer-targeted therapy (Sharma *et al*., 2024; Liu *et al*., 2024; Moosavi *et al*., 2021; Lai *et al*., 2021; Chang *et al*., 2024; Anitha *et al*., 2023; Yu *et al*., 2024). The findings from the above-mentioned studies also align with our current study, which delineates that c-MET/HGF signaling is involved in colon cancer propagation, and its overexpression leads to an increase in tumor stage and results in tumor metastasis (Bontoux *et al*., 2023; Liu *et al*., 2012a; Yao *et al*., 2019; Parizadeh *et al*., 2019).

In conclusion, our research indicated that the c-MET gene plays a substantial role in colon cancer propagation, invasion, and subsequent metastasis to various related organs. The c-MET/HGF axis, in addition, has been a significant contributor to tumor proliferation and aggressiveness so it can be a suitable target for targeted drug delivery and will open new and exciting opportunities for curbing metastasis and multidrug resistance in colon cancer (Mukae *et al*., 2020; Novoa-Díaz *et al*., 2022; Ji *et al*., 2025).

CONCLUSION

This study highlights the critical role of c-MET expression in the progression and metastasis of colon cancer, as demonstrated in both human and MNU-induced mouse models. The significant correlation between c-MET and HGF underscores their contribution to tumor invasion and proliferation, particularly in advanced stages. Diagnostic techniques, including qPCR, IHC, and protein assays, validated c-MET as a robust biomarker for identifying and characterizing colon cancer progression. These findings suggest that targeting the c-MET/HGF axis offers a promising therapeutic approach to reduce metastasis and improve treatment outcomes. This study also suggests that methyl nitroso urea-induced animal models can be the best choice for studying the targeted designed therapeutic drugs for the c-MET/HGF axis. Future research should explore population-specific therapeutic strategies to optimize colon cancer management.

DECLARATIONS

Acknowledgements

This research was supported by the School of Biochemistry and Biotechnology, University of Punjab, Lahore, Pakistan.

Funding

This work was supported by School of Biochemistry and Biotechnology, University of the Punjab, Lahore.

Institutional review board statement

The research experiments conducted in this article on both humans and mice were approved by the Ethics Committee of the Faculty of Life Sciences, University of the Punjab, Lahore, and Shaikh Zayed Hospital, Lahore, Pakistan.

Ethical approval

With the ethical approval of the Institutional Review Board (IRB) of Shaikh Zayed Hospital, Lahore, (Ref No: SZMC/IRB/210/2022) colon cancer tissue samples were collected after surgery, and the patient's written consent form was duly signed.

Generative AI and AI-assisted technology statement

The authors declare that no generative artificial intelligence (AI) tools or AI assisted technologies were used in the preparation, writing, data analysis or editing of this manuscript. All content is the result of the authors' own intellectual work and effort.

Statement of conflict of interest

The authors have declared no conflict of interest.

REFERENCES

Ahmad, R., Abbasi, H.J., Nasir, I.U.I. and Shah, M.F., 2024. Demographic characteristics and short-term outcomes of laparoscopic colon cancer surgeries at a newly developed cancer center in Peshawar, Pakistan. *Pak. J. med. Sci.,* **40**: 918-921. https://doi.org/10.12669/pjms.40.5.8732

Anitha, K., Dua, K., Chellappan, D.K., Gupta, G., Singh, S.K., Lakshmi, S.M. and Bhatt, S., 2023. HGF/c-Met: A potential target for the treatment of various cancers. *Curr. Enz. Inhibit.,* **19**: 71–80. https://doi.org/10.2174/1573408019666230227101036

Barzaman, K., Vafaei, R., Samadi, M., Kazemi, M.H., Hosseinzadeh, A., Merikhian, P., Moradi-Kalbolandi, S., Eisavand, M.R., Dinvari, H. and Farahmand, L., 2022. Anti-cancer therapeutic strategies based on HGF/Met, EpCAM, and tumor-stromal cross talk. *Cancer Cell Int.,* **22**. https://doi.org/10.1186/s12935-022-02658-z

Birchmeier, C., Birchmeier, W., Gherardi, E. and Vande, Woude, G.F., 2003. Met, metastasis, motility and more. *Nat. Rev. Mol. Cell Biol.*, **4**: 915–925. https://doi.org/10.1038/nrm1261

Bontoux, C., Hofman, V., Abboute, M., Lespinet-Fabre, V., Lalvée, S., Goffinet, S., Bordone, O., Long-Mira, E., Lassalle, S., Murcy, F., Rignol, G., Heeke, S., Ilie, M. and Hofman, P., 2023. C-met immunohistochemistry as reflex test at diagnosis for non-small cell lung cancer: A real-world experience from a monocentric case series. *J. Clin. Pathol.*, **78**: 35-41. https://doi.org/10.1136/jcp-2023-209202

Bradley, C.A., Dunne, P.D., Bingham, V., McQuaid, S., Khawaja, H., Craig, S., James, J., Moore, W.L., McArt, D.G., Lawler, M., Dasgupta, S., Johnston, P.G. and Van, Schaeybroeck, S., 2016. Transcriptional upregulation of C-MET is associated with invasion and tumor budding in colorectal cancer. *Oncotarget,* **7**: 78932–78945. https://doi.org/10.18632/oncotarget.12933

Bray, F., Laversanne, M., Sung, H., Ferlay, J., Siegel, R.L., Soerjomataram, I. and Jemal, A., 2024. Global cancer statistics 2022: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J. Clin.*, **74**: 229–263. https://doi.org/10.3322/caac.21834

Chang, Q., Huang, K., Zou, L., Li, A., Ye, Z., Lin, Q. and Gu, Y., 2024. Synthesis and evaluation of a novel C-Met-targeting cyclic peptide as a potential diagnostic agent for colorectal cancer. *Mol. Pharm.*, **21**: 3613–3622. https://doi.org/10.1021/acs.molpharmaceut.4c00330

Cleveland Clinic, 2022. *Colorectal (colon) cancer*. Retrieved from https://my.clevelandclinic.org/health/diseases/14501-colorectal-colon-cancer

Cruz, J., Reis-Filho, J.S., Silva, P. and Lopes, J.M., 2003. Expression of C-met tyrosine kinase receptor is biologically and prognostically relevant for primary cutaneous malignant melanomas. *Oncology*, **65**: 72–82. https://doi.org/10.1159/000071207

Cyr, D.P., Pun, C., Shivji, S., Mitrovic, B., Duan, K., Tomin, R., Sari, A., Brar, A., Zerhouni, S., Brar, M.S., Kennedy, E.D., Swallow, C.J., Kirsch, R. and Conner, J.R., 2023. Tumor budding assessment in colorectal carcinoma. *Am. J. Surg. Pathol.,* **48**: 251–265. https://doi.org/10.1097/PAS.0000000000002166

Danese, E. and Montagnana, M., 2017. Epigenetics of colorectal cancer: Emerging circulating diagnostic and prognostic biomarkers. *Annls Trans. Med.*, **5**: 279–279. https://doi.org/10.21037/atm.2017.04.45

Dong, Y., Xu, J., Sun, B., Wang, J. and Wang, Z., 2022. Met-targeted therapies and clinical outcomes: A systematic literature review. *Mol. Diagn. Amp. Ther.,* **26**: 203–227. https://doi.org/10.1007/s40291-021-00568-w

Faiella, A., Riccardi, F., Cartenì, G., Chiurazzi, M. and Onofrio, L., 2022. The emerging role of c-met in carcinogenesis and clinical implications as a possible therapeutic target. *J. Oncol.,* pp. 1–12. https://doi.org/10.1155/2022/5179182

García-Vilas, J.A. and Medina, M.Á., 2018. Updates on the hepatocyte growth factor/c-met axis in hepatocellular carcinoma and its therapeutic implications. *World J. Gastroenterol.,* **24**: 3695–

3708. https://doi.org/10.3748/wjg.v24.i33.3695

Ginty, F., Adak, S., Can, A., Gerdes, M., Larsen, M., Cline, H., Filkins, R., Pang, Z., Li, Q. and Montalto, M.C., 2008. The relative distribution of membranous and cytoplasmic met is a prognostic indicator in Stage I and II colon cancer. *Clin. Cancer Res.,* **14**: 3814–3822. https://doi.org/10.1158/1078-0432.CCR-08-0180

Han, Y., Yu, Y., Miao, D., Zhou, M., Zhao, J., Shao, Z., Jin, R., Le, X., Li, W. and Xia, Y., 2024. Targeting met in NSCLC: An ever-expanding territory. *JTO Clin. Res. Rep.,* **5**: 100630. https://doi.org/10.1016/j.jtocrr.2023.100630

Ji, M., Ganesan, S., Xia, B. and Huo, Y., 2025. Targeting C-m*et al*terations in cancer: A review of genetic drivers and therapeutic implications. *Cancers*, **17**: 1493. https://doi.org/10.3390/cancers17091493

Jin, F., Lin, Y., Yuan, W., Wu, S., Yang, M., Ding, S., Liu, J. and Chen, Y., 2024. Recent advances in c-Met based dual inhibitors in the treatment of cancers. *Eur. J. Med. Chem.,* **272**: 116477. https://doi.org/10.1016/j.ejmech.2024.116477

Kim, Y., Bang, S.S., Jee, S., Park, S., Shin, S.J. and Jang, K., 2020. Prevalence and clinicopathological significance of met overexpression and gene amplification in patients with gallbladder carcinoma. *Cancer Res. Treat.,* **52**: 481–491. https://doi.org/10.4143/crt.2019.370

Kim, Y.J., Choi, J.S., Seo, J., Song, J.Y., Eun, Lee, S., Kwon, M.J., Kwon, M.J., Kundu, J., Jung, K., Oh, E., Shin, Y.K. and Choi, Y.L., 2013. Met is a potential target for use in combination therapy with EGFR inhibition in triple-negative/basal-like breast cancer. *Int. J. Cancer*, **134**: 2424–2436. https://doi.org/10.1002/ijc.28566

Lai, X., Dong, Q., Xu, F., Wu, S., Yang, D., Liu, C., Li, Y., Li, Z. and Ma, D., 2021. Correlation of C-met expression with clinical characteristics and the prognosis of colorectal cancer. *J. Gastroint. Oncol.,* **12**: 2203–2210. https://doi.org/10.21037/jgo-21-536

Lee, M., Jain, P., Wang, F., Ma, P.C., Borczuk, A. and Halmos, B., 2021. M*et al*terations and their impact on the future of non-small cell lung cancer (NSCLC) targeted therapies. *Expert Opin. Therapeut. Targets,* **25**: 249–268. https://doi.org/10.1080/14728222.2021.1925648

Liu, N., Zheng, X., Yan, J., Jiang, A., Yao, Y. and He, W., 2024. Reversing met-mediated resistance in oncogene-driven NSCLC by Met-activated Wnt condensative prodrug. *Adv. Sci.*, **11**. https://doi.org/10.1002/advs.202400603

Liu, Y., Li. Q. and Zhu, L., 2012. Expression of the hepatocyte growth factor and c-met in colon cancer: Correlation with clinicopathological features and overall survival. *Tumori J.,* **98**: 105–112. https://doi.org/10.1177/030089161209800115

Liu, Y., Yu, X.F., Zou, J. and Luo, Z.H., 2015. Prognostic value of c-Met in colorectal cancer: A meta-analysis. *World J. Gastroenterol.,* **21**: 3706. https://doi.org/10.3748/wjg.v21.i12.3706

Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2 (-ΔΔC(T)) method. *Methods*, **25**: 402-408. https://doi.org/10.1006/meth.2001.1262

Lodhi, M.S., Khalid, F., Khan, M.T., Samra, Z.Q., Muhammad, S., Zhang, Y.J. and Mou, K., 2022. A novel method of magnetic nanoparticles functionalized with anti-folate receptor antibody and methotrexate for antibody mediated targeted drug delivery. *Molecules*, **27**: 261. https://doi.org/10.3390/molecules27010261

Lodhi, M.S., Khan, M.T., Aftab, S., Samra, Z.Q., Wang, H. and Wei, D.Q., 2021. A novel formulation of theranostic nanomedicine for targeting drug delivery to gastrointestinal tract cancer. *Cancer Nanotechnol.*, **12**. https://doi.org/10.1186/s12645-021-00098-4

Lodhi, M.S., Khan, M.T., Bukhari, S.M., Sabir, S.H., Samra, Z.Q., Butt, H. and Akram, M.S., 2021. Probing transferrin receptor overexpression in gastric cancer mice models. *ACS Omega*, **6**: 29893–29904. https://doi.org/10.1021/acsomega.1c04382

Moosavi, F., Giovannetti, E., Peters, G.J. and Firuzi, O., 2021. Combination of HGF/Met-targeting agents and other therapeutic strategies in cancer. *Crit. Rev. Oncol. Hematol.,* **160**: 103234. https://doi.org/10.1016/j.critrevonc.2021.103234

Mukae, Y., Miyata, Y., Nakamura, Y., Araki, K., Otsubo, A., Yuno, T., Mitsunari, K., Matsuo, T., Ohba, K. and Sakai, H., 2020. Pathological roles of c-met in bladder cancer: Association with cyclooxygenase-2, heme oxygenase-1, vascular endothelial growth factor-a and programmed death ligand 1. *Oncol. Lett.*, 20: 135–144. https://doi.org/10.3892/ol.2020.11540

Nagatsuma, A.K., Aizawa, M., Kuwata, T., Doi, T., Ohtsu, A., Fujii, H. and Ochiai, A., 2014. Expression profiles of HER2, EGFR, met and FGFR2 in a large cohort of patients with gastric adenocarcinoma. *Gastric Cancer,* **18**: 227–238. https://doi.org/10.1007/s10120-014-0360-4

Nakayama, G., Tanaka, C. and Kodera, Y., 2013.

Current options for the diagnosis, staging and therapeutic management of colorectal cancer. *Gastroint. Tumors,* **1**: 25–32. https://doi.org/10.1159/000354995

National Cancer Institute, 2024. *Colon cancer treatment health professional version*. Retrieved from https://www.cancer.gov/types/colorectal/hp/colon-treatment-pdq

Novoa-Díaz, M.B., Carriere, P., Gigola, G., Zwenger, A.O., Calvo, N. and Gentili, C., 2022. Involvement of met receptor pathway in aggressive behavior of colorectal cancer cells induced by parathyroid hormone-related peptide. *World J. Gastroenterol.*, **28**: 3177–3200. https://doi.org/10.3748/wjg.v28.i26.3177

Parizadeh, S.M., Jafarzadeh-Esfehani, R., Fazilat-Panah, D., Hassanian, S.M., Shahidsales, S., Khazaei, M., Parizadeh, S.M., Ghayour-Mobarhan, M., Ferns, G.A. and Avan, A., 2019. The potential therapeutic and prognostic impacts of the c-met/HGF signaling pathway in colorectal cancer. *IUBMB Life*, **71**: 802–811. https://doi.org/10.1002/iub.2063

Pennacchietti, S., Michieli, P., Galluzzo, M., Mazzone, M., Giordano, S. and Comoglio, P.M., 2003. Hypoxia promotes invasive growth by transcriptional activation of the met protooncogene. *Cancer Cell*, **3**: 347–361. https://doi.org/10.1016/S1535-6108(03)00085-0

Raghav, K., Morris, V., Tang, C., Morelli, P., Amin, H.M., Chen, K., Manyam, G.C., Broom, B., Overman, M.J., Shaw, K., Meric-Bernstam, F., Maru, D., Menter, D., Ellis, L.M., Eng, C., Hong, D. and Kopetz, S., 2016. Met amplification in metastatic colorectal cancer: An acquired response to EGFR inhibition, not a de novo phenomenon. *Oncotarget,* **7**: 54627–54631. https://doi.org/10.18632/oncotarget.10559

Rani, B., 2024. A wake-up call: The emerging crisis of colon cancer in young age in Pakistan. *J. Coll. Phys. Surg. Pak.*, **34**: 122-124. https://doi.org/10.29271/jcpsp.2024.01.122

Recondo, G., Che, J., Jänne, P.A. and Awad, M.M., 2020. Targeting MET dysregulation in cancer. *Cancer Discovery*, **10**: 922–934. https://doi.org/10.1158/2159-8290.CD-19-1446

Sharma, M., Strickler, J.H., Sommerhalder, D., Kuboki, Y., Perets, R., Cohen, J., Raimbourg, J., Nakajima, T.E., Yamamoto, N., Cruz-Correa, M., O'Neil, B., Ghiringhelli, F., Raghav, K.P., Li, R., Morrison, T.G., Neagu, Aristide, M.R., Freise, K.J., Biesdorf de Almeida, C., Vasilopoulos, A. and Powderly, J.D., 2024. First-in-human study of ABBV-400, a novel C-Met–targeting antibody-drug conjugate, in advanced solid tumors: Results in colorectal cancer. *J. Clin. Oncol.,* **42**(**16_suppl**): 3515–3515. https://doi.org/10.1200/JCO.2024.42.16_suppl.3515

Siegel, R.L., Giaquinto, A.N. and Jemal, A., 2024. Cancer statistics, 2024. *CA: Cancer J. Clin.*, **74**: 12–49. https://doi.org/10.3322/caac.21820

Sierra, J.R. and Tsao, M.S., 2011. c-MET as a potential therapeutic target and biomarker in cancer. Therapeutic. *Adv. Med. Oncol.,* **3**(1_suppl). https://doi.org/10.1177/1758834011422557

Sun, Y., Liu, W., Ma, G., Gao, D., Jiang, Y., Liu, Q. and Du, J., 2013. Expression of HGF and met in human tissues of colorectal cancers: Biological and clinical implications for synchronous liver metastasis. *Int. J. Med. Sci.*, **10**: 548–559. https://doi.org/10.7150/ijms.5191

Sung, H., Ferlay, J., Siegel, R.L., Laversanne, M., Soerjomataram, I., Jemal, A. and Bray, F., 2021. Global cancer statistics 2020: Globocan estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: Cancer J. Clin.*, **71**: 209–249. https://doi.org/10.3322/caac.21660

Tang, C., Cortez, M.A., Hong, D. and Welsh, J.W., 2015. Targeting the c-Met kinase. In: *Targeted therapy of translational cancer research* (ed. W.Y. Cheung). John Wiley & Sons / Wiley-Blackwell. pp. 341–346. https://doi.org/10.1002/9781118468678.ch34

Terlecka, P., Krawczyk, P., Grenda, A. and Milanowski, J., 2021. Met gene dysregulation as a promising therapeutic target in lung cancer. A review. *J. Personal. Med.,* **11**: 1370. https://doi.org/10.3390/jpm11121370

Uchikawa, E., Chen, Z., Xiao, G.Y., Zhang, X. and Bai, X., 2021. Structural basis of the activation of c-Met receptor. *Nat. Commun.,* **12**. https://doi.org/10.1038/s41467-021-24367-3

Ueno, H., Kajiwara, Y., Shimazaki, H., Shinto, E., Hashiguchi, Y., Nakanishi, K., Maekawa, K., Katsurada, Y., Nakamura, T., Mochizuki, H., Yamamoto, J. and Hase, K., 2012. New criteria for histologic grading of colorectal cancer. *Am. J. Surg. Pathol.,* **36**: 193–201. https://doi.org/10.1097/PAS.0b013e318235edee

Wang, H., Rao, B., Lou, J., Li, J., Liu, Z., Li, A., Cui, G., Ren, Z. and Yu, Z., 2020. The function of the HGF/c-met axis in hepatocellular carcinoma. *Front. Cell Dev. Biol.*, **8**: 1-13. https://doi.org/10.3389/fcell.2020.00055

World Health Organization, 2023. *Colorectal cancer*. https://www.who.int/news-room/fact-sheets/detail/

colorectal-cancer?utm_source=chatgpt.com

Wu, J., Chen, J., Xi, Y., Wang, F., Sha, H., Luo, L., Zhu, Y., Hong, X. and Bu, S., 2018. High glucose induces epithelial-mesenchymal transition and results in the migration and invasion of colorectal cancer cells. *Exp. Therapeut. Med*., https://doi.org/10.3892/etm.2018.6189

Yao, J., Li, X., Yan, L., He, S., Zheng, J., Wang, X., Zhou, P., Zhang, L., Wei, G. and Sun, X., 2019. Role of HGF/c-met in the treatment of colorectal cancer with liver metastasis. *J. Biochem. mol. Toxicol.,* **33**. https://doi.org/10.1002/jbt.22316

Yu, Q.X., Fu, P.Y., Zhang, C., Li, L. and Huang, W.T., 2024. Mesenchymal-epithelial transition factor ampliification correlates with adverse pathological features and poor clinical outcome in colorectal cancer. *World J. Gastroint. Surg.,* **16**: 1395–1406. https://doi.org/10.4240/wjgs.v16.i5.1395