中国临床研究 Chin J Clin Res, February 2026, Vol.39, No.2

Cite as: Jing TC, Ma SX, Li YX, Dai XX, Niu SM, Ma HW. Expression and clinical significance of isoprenyl carboxyl methyltransferase in gastric cancer tissues [J]. Chin J Clin Res, 2026, 39 (2):188-192.

DOI: 10.13429/j.cnki.cjcr.2026.02.005

Expression and clinical significance of isoprenyl carboxyl methyltransferase in gastric cancer tissues

JING Tiancong*, MA Shangxian, LI Yuanxiao, DAI Xingxing, NIU Shaomin, MA Hanwei

**Second Clinical Medical College of Lanzhou University, Lanzhou, Gansu 730030, China*

Corresponding author:MA Hanwei, E-mail: mahanwei_xjtu@163. com

Abstract: Objective To study the expression level of isoprenyl carboxyl methyltransferase (ICMT)in gastric cancer and its effect on disease progression. **Methods** A clinical retrospective study was conducted on 60 patients diagnosed with gastric cancer who underwent surgical treatment at Lanzhou University Second Hospital between May and September 2022. The expression levels of ICMT mRNA and protein in tumor tissue and adjacent tissue were compared using immunohistochemistry (IHC) and polymerase chain reaction (PCR) techniques. The correlation between ICMT expression levels and patient clinical characteristics was analyzed. **Results** IHC showed structural disorganization of tumor tissue，scattered cell arrangement，and loss of polarity. The number of positive ICMT protein granules in gastric tumor tissue was significantly higher than that in adjacent tissues. PCR showed that the expression levels of ICMT mRNA in gastric tumor tissue were significantly higher than those in adjacent tissue (P<0.05). Correlation analysis with clinical features showed that the proportion of gastric cancer patients having high expression of ICMT mRNA presented a distribution trend from high to low in the following clinical characteristics，with statistically significant differences. (1)Cancer locations：gastric corpus cancer > cardiac cancer > gastric antrum cancer (χ^2 = 7.161，P=0.028); (2) Lauren' s classification: mixed > intestinal > diffuse (χ^2=13. 153, P=0.001); (3) grades of differentiation: poorly differentiated > moderately to well differentiated (χ^2=7. 625, P=0.006); (4)T stage: (T3+T4) stage > (T1+T2) stage (χ^2= 5. 740, P= 0.017); (5)p53 expression (based on the conclusions of the patients pathology reports): mutated type > wild type (χ^2= 5. 831, P=0.016). **Conclusion** ICMT is highly expressed in gastric cancer, and the difference in expression is associated with the location of the lesion, degree of differentiation, and stage of gastric cancer. p53 mutation may be the basis for the high expression of ICMT in gastric tumor tissue.

Keywords: Isoprenyl carboxyl methyltransferase; Rat sarcoma protein; Gastric cancer; p53; Target

Fund program: Natural Science Foundation of Gansu Province (22JR5RA958); Scientific and Technological Innovation Program for Talentof the Second Hospital of Lanzhou University (CY2021-QN-B07); Research and Cultivation Plan for Excellent Students (CYXZ2022-20)

Rat sarcoma (Ras) protein is an important intracellular signal transduction molecule that regulates various biological processes including cell growth, differentiation, and survival. Once Ras mutates, it can induce a variety of human tumors. More than 30% of malignant tumors are caused by Ras gene mutations [1], and Ras mutations often indicate higher tumor malignancy and poor clinical prognosis [2]. Isoprenylcysteine carboxyl methyltransferase (ICMT) is the sole key enzyme that catalyzes the methylation of the C-terminal isoprenylcysteine residue of CaaX proteins, including Ras proteins. It plays an essential role in the oncogenic mutation of all Ras isoforms and tumor maintenance [1]. Previous studies have found that overexpression of ICMT in bladder cancer [3], oropharyngeal cancer [4], and other tumors is closely related to the malignant biological characteristics of tumors, especially in tumor invasion. Since the discovery of spermatinamine, the first natural product inhibitor of ICMT, studies targeting ICMT for the treatment of colorectal cancer [5], pancreatic cancer [6], and other malignancies have been reported successively. Because inhibition of ICMT can block the isoprenylation process and interfere with the activation of the Ras pathway, ICMT is expected to become a novel antitumor target [1].

Gastric cancer remains one of the major life-threatening malignant tumors worldwide. There are nearly 1 million new cases each year, resulting in more than 650,000 deaths [7]. As one of the pathogenic mechanisms of gastric cancer [8], Ras mutation has been confirmed in studies of gastric cancer specimens [9]. In this study, by analyzing the clinical data of gastric cancer patients and comparing the expression differences of ICMT between gastric cancer tissues and adjacent normal tissues, we evaluated the influence of ICMT on the disease progression of gastric cancer, aiming to explore a new direction for the prevention and treatment of gastric cancer in the future.

1 Materials and Methods

1.1 Study Subjects

Clinical data and surgical specimens were retrospectively collected from 60 patients diagnosed with gastric cancer and undergoing surgical treatment at the Second Hospital of Lanzhou University from May to September 2022. All enrolled patients met the diagnostic criteria for gastric cancer. Clinicopathological staging was performed according to the 8th edition of the TNM staging system issued by the American Joint Committee on Cancer (AJCC) and the Union for International Cancer Control (UICC) in 2016 [10]. Patients with other malignant tumors, mental illness, emotional instability, or inability to cooperate were excluded. All enrolled patients had not received radiotherapy or chemotherapy preoperatively, with complete clinical data, and all signed informed consent forms. The study was approved by the Medical Ethics Committee of the Second Hospital of Lanzhou University

(2023A-068).

1.2 Specimen Collection

Within 15 minutes after resection of gastric cancer specimens, appropriate cancer tissues were taken from non-hemorrhagic and non-necrotic areas of the tumor, and adjacent normal tissues were obtained from the surgical margin (3–5 cm away from the cancerous lesion). Tissues were immediately placed in pre-numbered cryotubes and stored at −80 °C until use. Sections for immunohistochemistry (IHC) were borrowed from the surgical specimen bank of the Department of Pathology, the Second Hospital of Lanzhou University.

1.3 Materials and Methods

1.3.1 IHC Staining

Paraffin sections were sequentially subjected to degreasing, dewaxing, rehydration, and antigen retrieval. Sections were incubated in 10% hydrogen peroxide solution at room temperature in the dark for 20 min. After blocking with 5% bovine serum albumin at room temperature for 1 h, the blocking solution was gently removed, and ICMT antibody (GeneTex, GTX129471) was added, followed by incubation at 4 °C overnight. On the next day, sections were washed with phosphate-buffered saline, incubated with goat anti-rabbit IgG secondary antibody (Abbkine, A21020) at room temperature for 50 min, and then treated with diaminobenzidine chromogenic solution (Beyotime, P0203). Harris hematoxylin was used for counterstaining, followed by dehydration and mounting. The Tissue FAXS Plus panoramic tissue quantitative analysis system (Tissue Gnostics Asia Pacific) was used to observe and capture images. Five visual fields were randomly selected from each sample. Image-Pro Plus software was used to analyze the images and calculate the mean optical density of positive particles (brownish yellow) in each field. The average value was used to reflect the expression intensity of the target protein.

1.3.2 Polymerase Chain Reaction (PCR)

Total RNA extraction:Gastric cancer and adjacent tissues stored at −80°C were quickly transferred to centrifuge tubes containing appropriate RNA extraction solution, and homogenized on ice using a tissue grinder. Chloroform was then added, followed by vigorous shaking for 15 s and lysis on ice for 30 min. After centrifugation at 4 °C for 15 min, the clear upper liquid was transferred to a new centrifuge tube, mixed with an equal volume of isopropanol, and allowed to stand for 10 min, followed by another centrifugation for 10 min. The RNA precipitate at the bottom was washed with 75% ethanol, centrifuged for 5 min, and this step was repeated 1–2 times. Ethanol was discarded, and the tube was inverted for air-drying. Then 20–40 μL diethyl pyrocarbonate-treated water (Biosharp, 143198) was added. The purity and concentration of the extracted RNA were detected using a micro nucleic acid detector (Thermo Scientific, Nanodrop 2000).

RNA reverse transcription: According to the instructions of the RNA reverse transcription kit (SparkJade, AG0304-B), the reaction mixture was prepared on ice, added to RNA samples, and placed in a conventional PCR instrument (BioGener, GE4852T). Then 10 μL SPARK script II RT Plus Master Mix was added, and reverse transcription was performed to obtain cDNA. The experiment was independently repeated three times.

Fluorescent quantitative real-time PCR amplification of cDNA:Based on previous reports [11], primer sequences used in this study were designed as follows:

ICMT forward primer: 5′-CGC TTG GTT TCG GCA TCC TTC T-3′; ICMT reverse primer: 5′-CGG AAG AAT CGC CAC ACT GTC A-3′; GAPDH forward primer: 5′-TGC ACC ACC AAC TGC TTA GC-3′; GAPDH reverse primer: 5′-AGC TCA GGG ATG ACC TTG CC-3′

Reagents and primers were added to amplification tubes according to the reaction system of the cDNA amplification kit (SparkJade, AH0104-B). After centrifugation and mixing, amplification was performed on a real-time fluorescent quantitative PCR instrument (QIAGEN, Rotor-Gene Q). The experiment was independently repeated three times.

The relative expression level of ICMT mRNA in gastric cancer tissues and adjacent normal tissues was calculated using the $2^{-\Delta\Delta Ct}$ method. Compared with the adjacent normal tissue group, ICMT mRNA with a $2^{-\Delta\Delta Ct}$ value > 1 was defined as high expression, and < 1 as low expression.

1.4 Statistical Analysis

GraphPad Prism 8 software was used for data analysis and graphing. Count data were expressed as cases (%). The χ^2 test was used to analyze the association between ICMT expression and clinical characteristics of gastric cancer patients. A value of $P < 0.05$ was considered statistically significant.

2 Results

2.1 ICMT Protein Expression in Two Types of Tissues

IHC was used to observe the expression of ICMT protein in cancerous and adjacent normal tissues of gastric cancer patients. As shown in **Figure 1**, gastric cancer tissues exhibited disordered structure, irregular cell arrangement, and loss of polarity. The number of positive ICMT particles in the cytoplasm was significantly higher than that in adjacent normal tissues.

2.2 ICMT mRNA Expression in Two Types of Tissues

PCR was used to compare the relative mRNA

expression levels of ICMT in gastric cancer tissues and adjacent normal tissues from 60 patients. As shown in **Figure 2**, the expression level of ICMT in gastric cancer tissues was significantly higher than that in adjacent normal tissues ($P < 0.05$).

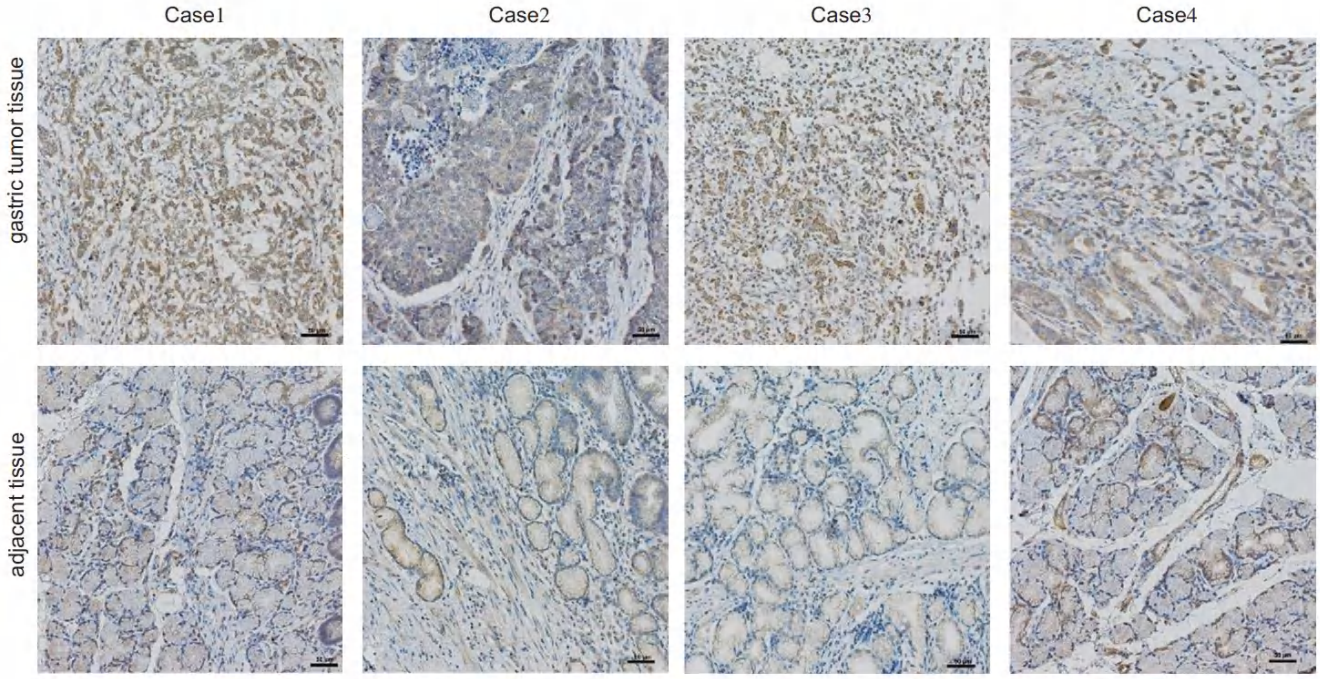


Fig.1 The expression level of ICMT protein in gastric tumor tissue and adjacent tissue

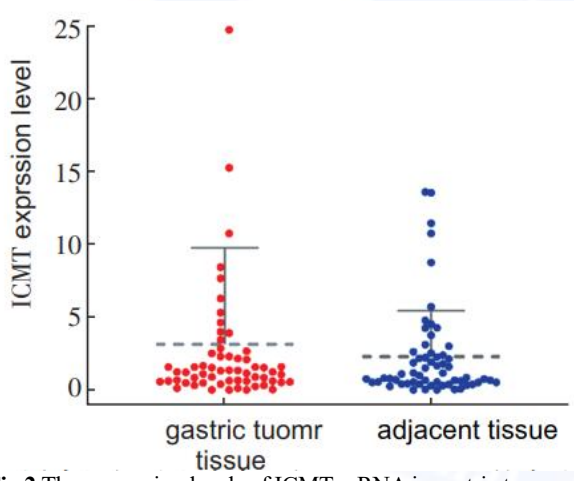


Fig.2 The expression levels of ICMT mRNA in gastric tumor tissue and adjacent tissue

2.3 Association Between ICMT mRNA Expression and Clinical Characteristics of Gastric Cancer Patients

In gastric cancer tissues, 34 cases (56. 67%) showed high ICMT expression and 26 cases (43. 33%) low expression. In adjacent normal tissues, 22 cases (36. 67%) showed high ICMT expression and 38 cases (63. 33%) low expression. The proportion of high ICMT expression in gastric cancer tissues was significantly higher than that in adjacent normal tissues, with a statistically significant difference ($\chi^2 = 4.821$, $P = 0.028$).

Analysis of clinical characteristics see Table 1.

(1) Tumor location: The expression difference of ICMT among different gastric cancer locations was statistically significant ($\chi^2 = 7.161$, $P = 0.028$). The highest proportion of high ICMT expression was found in gastric body cancer, followed by cardiac cancer, and the lowest in antral cancer. (2) Histological features: ICMT expression was significantly associated with Lauren classification ($\chi^2 = 13.153$, $P = 0.001$). The highest proportion of high ICMT expression was observed in mixed-type gastric cancer, followed by intestinal type, and the lowest in diffuse type. (3) Differentiation grade: ICMT level in poorly differentiated cancer tissues was significantly higher than that in moderately/well-differentiated group ($\chi^2 = 7.625$, $P = 0.006$). (4) TNM stage, metastasis, and invasion: According to T stage (primary tumor and invasion), ICMT expression was significantly higher in gastric cancer tissues at T3+T4 stage ($\chi^2 = 5.740$, $P = 0.017$). However, ICMT expression showed no significant correlation with N stage (lymph node involvement), M stage (distant metastasis), vascular invasion, or neural invasion ($P > 0.05$).

(5)p53 expression: The proportion of high ICMT expression in patients with mutant p53 was significantly higher than that in patients with wild-type p53 ($\chi^2 = 5.831$, $P = 0.016$).

Tab.1 Correlation between the expression levels of ICMT mRNA and clinical characteristics in gastric cancer patients ［case (%)］

Item	Total Cases	ICMT High Expression (n=34)	ICMT Low Expression (n=26)	χ^2	*P*
Gender				0.184	0.668
Male	41	24 (58.54)	17 (41.46)		
Female	19	10 (52.63)	9 (47.37)		
Age				0.102	0.750
<60 years	24	13 (54.17)	11 (45.83)		
≥60 years	36	21 (58.33)	15 (41.67)		
Tumor Size				1.778	0.182
< 5 cm	22	10 (45.45)	12 (54.55)		
≥ 5 cm	38	24 (63.16)	14 (36.84)		
Lesion Site				7.161	0.028
Upper stomach (Cardia)	19	12 (63.16)	7 (36.84)		
Middle stomach (Corpus)	15	12 (80.00)	3 (20.00)		
Lower stomach (Antrum)	26	10 (38.46)	16 (61.54)		
Infiltration Depth				0.477	0.450
Submucosa-muscularis propria	19	12 (63.16)	7 (36.84)		
Serosa-entire layer	41	22 (53.66)	19 (46.34)		
Histological Type of Gastric Cancer				0.037	0.194
Adenocarcinoma	58	33 (56.90)	25 (43.10)		
Signet ring cell carcinoma	2	1 (50.00)	1 (50.00)		
Lauren Classification				13.153	0.001
Diffuse type	17	4 (23.53)	13 (76.47)		
Intestinal type	18	10 (55.56)	8 (44.44)		
Mixed type	25	20 (80.00)	5 (20.00)		
Differentiation Degree				7.625	0.006
Poorly differentiated	47	31 (65.96)	16 (34.04)		
Moderately/well differentiated	13	3 (23.08)	10 (76.92)		
T Stage				5.740	0.017
T1+T2	16	5 (31.25)	11 (68.75)		
T3+T4	44	29 (65.91)	15 (34.09)		
N Stage				0.045	0.832
N0	17	10 (58.82)	7 (41.18)		
N1~3	43	24 (55.81)	19 (44.19)		
M Stage				1.750	0.186
M0	56	33 (58.93)	23 (41.07)		
M1	4	1 (25.00)	3 (75.00)		
Vascular Invasion				1.922	0.166
Yes	51	27 (52.94)	24 (47.06)		
No	9	7 (77.78)	2 (22.22)		
Nerve Invasion				1.922	0.166
Yes	51	27 (52.94)	24 (47.06)		
No	9	7 (77.78)	2 (22.22)		
p53 Expression in Cancer Tissue				5.831	0.016
Wild-type	22	8 (36.36)	14 (63.64)		
Mutant type	38	26 (68.42)	12 (31.58)		

3 Discussion

ICMT is a key enzyme in the Ras signaling pathway, and the specific methylation event it participates in represents the final step of post-translational modification of Ras protein. Previous studies have confirmed that inhibition of ICMT can improve the malignant phenotype of tumors.

Data from The Cancer Genome Atlas database show that the most common type of Ras mutation in gastric cancer is K-Ras mutation [12]. Peng et al. [13] detected 126 gastric cancer tissue samples and 9 plasma samples using Nested and COLD-PCR, and reported a K-Ras mutation rate of 6. 67%. Another study indicated that K-Ras mutation may be involved in the early carcinogenesis of differentiated gastric cancer [14]. The present study demonstrated that both mRNA and protein expression levels of ICMT were significantly higher in gastric cancer tissues than in adjacent normal tissues. The proportion of high ICMT expression was significantly higher in poorly differentiated gastric cancer than in moderately/well-differentiated cancer, and significantly lower in T1+T2 stage than in T3+T4 stage. These results suggest that upregulated ICMT expression is a molecular feature in gastric cancer patients and is closely related to tumor malignancy and disease progression. Combined with the molecular characteristics and functional features of ICMT, we propose that the ICMT gene may be an oncogene associated with gastric cancer, which can promote the occurrence and progression of gastric cancer by regulating its downstream effector molecules.

Ma et al. [15] retrospectively analyzed the endoscopic findings of 6,446 Chinese gastric cancer patients and found that the proportion of gastric cancer located in the cardia and fundus was as high as 50.5%. A large-sample case-control study conducted by Yang et al. [16] showed that 78. 5% of non-cardiac cancers and 62. 1% of cardiac cancers could be attributed to Helicobacter pylori (Hp) infection. Hp mainly colonizes the gastric antrum and can also reach the cardia via gastroesophageal reflux to induce disease. Various virulence factors produced by Hp can activate multiple signaling pathways including Ras [8, 17], damage the mucosa, trigger inflammation, and even lead to carcinogenesis. Although only 19 cases of cardiac cancer were included in this study, 12 of them showed high-level ICMT protein expression, suggesting that ICMT may be involved in the pathogenesis of cardiac cancer.

Mutation of the tumor suppressor gene p53 is one of the common somatic events in human malignant tumors. Mutant p53 participates in tumorigenesis and progression by acquiring novel activities through diverse mechanisms [18–19]. Mutant p53 can alter gene expression in the mevalonate pathway [20], and ICMT is an important metabolic enzyme in this pathway, a key gene encoding a methyltransferase, and the final enzyme in protein isoprenylation. p53 status affects ICMT mRNA and protein levels: mutant p53

promotes ICMT expression [18], whereas wild-type p53 exerts the opposite effect, because wild-type p53 inhibits the region from −209 to −14 of the ICMT promoter. Once p53 mutates, this inhibitory effect is abolished, thereby synergizing with increased ICMT expression [21]. This promotes isoprenylation of key proteins in tumorigenesis (such as Ras) at multiple levels and enhances the malignant biological behavior of tumors. This conclusion was further confirmed by comparing the correlation between p53 status and ICMT expression in breast cancer and lung cancer [21]. Our study found that gastric cancer with mutant p53 showed a higher proportion of high ICMT expression, consistent with the above findings. Disruption of the balance between tumor suppressor genes and proto-oncogenes is critical for tumorigenesis. The tumor suppressor p53 and oncogene Ras are considered determinants of cell fate, with complex crosstalk including mutual regulation or coordinated modulation of key tumor-related genes [19]. Since ICMT is one of the key enzymes for Ras activation, we hypothesize that mutation of the tumor suppressor p53 and overactivation of Ras may constitute the molecular basis for high ICMT expression, possibly through synergistic interaction. However, the exact regulatory mechanism requires further investigation.

In conclusion, this study analyzed the correlation between ICMT protein expression and clinical and pathological characteristics of gastric cancer patients. We found that ICMT is highly expressed in gastric cancer, and its expression is associated with tumor location, differentiation grade, and disease stage. p53 mutation may be the pathological basis for high ICMT expression in gastric cancer, which may provide a new perspective for the prevention and treatment of gastric cancer in the future.

Conflict of interest None

Reference

[1] Sabt A, Tawfik HO, Khaleel EF, et al. An overview of recent advancements in small molecules suppression of oncogenic signaling of K-RAS: an updated review[J]. Mol Divers, 2024, 28 (6): 4581-4608.
[2] Chen K, Zhang YL, Qian L, et al. Emerging strategies to target RAS signaling in human cancer therapy[J]. J Hematol Oncol, 2021, 14 (1): 116.
[3] Luo X, Xie FM, Qin GQ, et al. circICMT upregulates and suppresses the malignant behavior of bladder cancer[J]. Transl Oncol, 2025, 52: 102262.
[4] Masago K, Kuroda H, Sasaki E, et al. Novel gene fusions in human oropharyngeal carcinoma[J]. Cancer Genet, 2024, 286/287: 29-34.
[5] Mouhcine M，Kadil Y，Segmani I，et al. In silicoexploration of a novel ICMT inhibitor with more solubility than cysmethynil against membrane localization of *KRAS* mutant in colorectal cancer［J］.Curr Comput Aided Drug Des, 2024, 20 (7): 1055-1069.
[6] Zhao JX, Zhao ZQ, Hou WT, et al. Quantitative proteomics explore the potential targets and action mechanisms of hydroxychloroquine[J]. Molecules, 2022, 27 (16): 5175.
[7] Sundar R, Nakayama I, Markar SR, et al. Gastric cancer[J]. Lancet, 2025, 405 (10494): 2087-2102.
[8] Morgos DT, Stefani C, Miricescu D, et al. Targeting PI3K/AKT/mTOR and MAPK signaling pathways in gastric cancer[J]. Int J Mol Sci, 2024, 25 (3): 1848.
[9] Huang Y, Wei J, Liu BR. Research advances of K-ras mutation in the prognosis and targeted therapy of gastric cancer[J]. Chin J Oncol, 2016, 38 (2): 81-85. [**In Chinese]**
[10] Amin MB, Edge SB, Greene FL. AJCC Cancer Staging Manual [M]. 8th ed. New York: Springer, 2016: 203-220.
[11] Li YJ, Gao XR, Yang CC, et al. CircRNA hsa_circ_0018289 exerts an oncogenic role in cervical cancer progression through miR-1294/ICMT axis[J]. J Clin Lab Anal, 2022, 36 (5): e24348.
[12] Chen SJ, Li FY, Xu D, et al. The function of RAS mutation in cancer and advances in its drug research[J]. Curr Pharm Des, 2019, 25 (10): 1105-1114.
[13] Peng NQ, Zhao XT. Comparison of K-ras mutations in lung, colorectal and gastric cancer[J]. Oncol Lett, 2014, 8 (2): 561-565.
[14] Hiyama T, Haruma K, Kitadai Y, et al. K-ras mutation in *Helicobacter pylori*-associated chronic gastritis in patients with and without gastric cancer[J]. Int J Cancer, 2002, 97 (5): 562-566.
[15] Ma T, Sui Y, Lu JH, et al. Retrospective analysis of the characteristics of lesions in 6446 cases of gastric cancer under endoscopy[J]. Mod Dig Interv, 2022, 27 (6): 702-706. [**In Chinese]**
[16] Yang L, Kartsonaki C, Yao P, et al. The relative and attributable risks of cardia and non-cardia gastric cancer associated with *Helicobacter pylori* infection in China: a case-cohort study[J]. Lancet Public Health, 2021, 6 (12): e888-e896.
[17] Alipour M. Molecular mechanism of *Helicobacter pylori*-induced gastric cancer[J]. J Gastrointest Cancer, 2021, 52 (1): 23-30.
[18] Borini Etichetti CM, Arel Zalazar E, Cocordano N, et al. Beyond the mevalonate pathway: control of post-prenylation processing by mutant p53[J]. Front Oncol, 2020, 10: 595034.
[19] Yuan M, Zhu XY, Zheng JY, et al. Epstein-Barr virus infection and expression of p53 protein and their clinicopathological features in gastric cancer[J]. Chin J Metastatic Cancer, 2024, 7 (2): 179-183. [**In Chinese]**
[20] Freed-Pastor WA, Mizuno H, Zhao X, et al. Mutant p53 disrupts mammary tissue architecture *via* the mevalonate pathway[J]. Cell, 2012, 148 (1/2): 244-258.
[21] Borini Etichetti C, Di Benedetto C, Rossi C, et al. Isoprenylcysteine carboxy methyltransferase (ICMT) is associated with tumor aggressiveness and its expression is controlled by the p53 tumor suppressor[J]. J Biol Chem, 2019, 294 (13): 5060-5073.

Submission received: 2025-04-16/ **Revised:** 2025-06-26

·消化道肿瘤专题·论著·

异戊二烯羧基甲基转移酶在胃癌组织中的表达与临床意义

景天聪[1]，马尚贤[2]，李元枭[3]，戴星星[3]，牛少敏[4]，马汉伟[3]

1. 兰州大学第二临床医学院，甘肃 兰州 730030；2. 甘肃省人民医院普外二科，甘肃 兰州 730000；
3. 兰州大学第二医院小儿消化科，甘肃 兰州 730030；4. 兰州大学第二医院小儿心血管科，甘肃 兰州 730030

摘要：目的　研究异戊二烯羧基甲基转移酶(ICMT)在胃癌中的表达水平及对疾病进程的影响。**方法**　回顾性收集兰州大学第二医院2022年5月至9月确诊为胃癌并行手术治疗的60例患者的临床资料及手术标本，通过免疫组织化学法(IHC)及聚合酶链式反应(PCR)技术，对比癌组织和癌旁组织中ICMT mRNA和蛋白的表达水平，并分析ICMT表达水平与患者临床特征之间的关联性。**结果**　IHC结果显示，癌组织结构紊乱、细胞排列杂乱、极向丧失，胞浆中ICMT蛋白的阳性颗粒数显著高于癌旁正常组织。PCR结果显示，ICMT mRNA在胃癌组织的表达水平显著高于癌旁组织($P<0.05$)。与临床特征的关联性分析显示，胃癌患者中高表达ICMT mRNA的占比均呈现由高到低的分布趋势，且差异具有统计学意义：(1) 病变部位，胃体癌>贲门癌>胃窦癌($\chi^2=7.161$，$P=0.028$)；(2) Lauren分型，混合型>肠型>弥漫型($\chi^2=13.153$，$P=0.001$)；(3) 分化程度，低分化>中高分化($\chi^2=7.625$，$P=0.006$)；(4) T分期，T3+T4期>T1+T2期($\chi^2=5.740$，$P=0.017$)；(5) p53蛋白表达(基于病理结论)，突变型>野生型($\chi^2=5.831$，$P=0.016$)。**结论**　ICMT在胃癌患者中高表达，且表达差异与胃癌的病变部位、分化程度、病程阶段存在关联，*p53*基因突变可能是胃癌组织ICMT高表达的病理基础。

关键词：异戊二烯羧基甲基转移酶；大鼠肉瘤蛋白；胃癌；*p53*基因；靶点
中图分类号：R735.2　**文献标识码：**A　**文章编号：**1674-8182(2026)02-0188-05

Expression and clinical significance of isoprenyl carboxyl methyltransferase in gastric cancer tissues

JING Tiancong*, MA Shangxian, LI Yuanxiao, DAI Xingxing, NIU Shaomin, MA Hanwei

*Second Clinical Medical College of Lanzhou University, Lanzhou, Gansu 730030, China

Corresponding author: MA Hanwei, E-mail: mahanwei_xjtu@163.com

Abstract: Objective　To study the expression level of isoprenyl carboxyl methyltransferase (ICMT) in gastric cancer and its effect on disease progression. **Methods**　A clinical retrospective study was conducted on 60 patients diagnosed with gastric cancer who underwent surgical treatment at Lanzhou University Second Hospital between May and September 2022. The expression levels of ICMT mRNA and protein in tumor tissue and adjacent tissue were compared using immunohistochemistry (IHC) and polymerase chain reaction (PCR) techniques. The correlation between ICMT expression levels and patient clinical characteristics was analyzed. **Results**　IHC showed structural disorganization of tumor tissue, scattered cell arrangement, and loss of polarity. The number of positive ICMT protein granules in gastric tumor tissue was significantly higher than that in adjacent tissues. PCR showed that the expression levels of ICMT mRNA in gastric tumor tissue were significantly higher than those in adjacent tissue ($P<0.05$). Correlation analysis with clinical features showed that the proportion of gastric cancer patients having high expression of ICMT mRNA presented a distribution trend from high to low in the following clinical characteristics, with statistically significant differences. (1) Cancer locations: gastric corpus cancer > cardiac cancer > gastric antrum cancer ($\chi^2=7.161$, $P=0.028$); (2) Lauren's

DOI:10.13429/j.cnki.cjcr.2026.02.005
基金项目：甘肃省自然科学基金资助项目(22JR5RA958)；兰州大学第二医院“萃英科技创新”计划资助项目(CY2021-QN-B07)；2022年萃英学子科研培育计划(CYXZ2022-20)
通信作者：马汉伟，E-mail：mahanwei_xjtu@163.com
网络出版日期：2025-11-19
网络出版地址：https://link.cnki.net/urlid/32.1811.R.20251118.1358.002

QR code for English version

classification: mixed > intestinal > diffuse (χ^2=13.153, P=0.001); (3) grades of differentiation: poorly differentiated > moderately to well differentiated (χ^2=7.625, P=0.006); (4) T stage: (T3+T4) stage >(T1+T2) stage (χ^2= 5.740, P=0.017); (5) p53 protein expression (based on the conclusions of the patients' pathology reports): mutated type > wild type (χ^2=5.831, P=0.016). **Conclusion** ICMT is highly expressed in gastric cancer, and the difference in expression is associated with the location of the lesion, degree of differentiation, and stage of gastric cancer. *p53* mutation may be the basis for the high expression of ICMT in gastric tumor tissue.

Keywords: Isoprenyl carboxyl methyltransferase; Rat sarcoma protein; Gastric cancer; *p53* gene; Target

Fund program: Natural Science Foundation of Gansu Province (22JR5RA958); "Cuiying Scientific and Technological Innovation" Program of Lanzhou University Second Hospital (CY2021-QN-B07); 2022 Cuiying Students Scientific Research Training Program (CYXZ2022-20)

大鼠肉瘤(rat sarcoma, Ras)蛋白作为一种细胞内重要的信号转导分子,调节细胞生长、分化、生存等生物学过程。*Ras*一旦发生突变,可能诱发多种人类肿瘤。超过30%的恶性肿瘤是由*Ras*基因突变引起的[1],且*Ras*突变常预示着肿瘤的恶性程度更高、临床预后差[2]。异戊二烯羧基甲基转移酶(isoprenyl carboxyl methyltransferase, ICMT)是催化包括Ras蛋白在内的CaaX蛋白C端异戊二烯基半胱氨酸残基的甲基化过程的唯一关键酶,其在所有Ras亚型的恶性突变和肿瘤维持中发挥重要作用[1]。既往研究发现,膀胱癌[3]、口咽癌[4]等肿瘤中ICMT的过度表达与肿瘤的恶性生物学特性密切相关,特别在肿瘤的侵袭性特征中尤为突出。自第一个ICMT天然产物抑制剂spermatinamine被发现以来,靶向ICMT作为治疗结直肠癌[5]、胰腺癌[6]等研究被相继报道。基于抑制ICMT可阻断异戊二烯化进程,干扰Ras途径的激活,因此ICMT有望成为一种新的抗肿瘤靶点[1]。

胃癌目前仍然是世界范围内威胁生命的重大恶性肿瘤之一。每年有近100万新发病例,导致超过65万人死亡[7]。*Ras*突变作为胃癌的致病机制之一[8],已在胃癌标本研究中得到证实[9]。本研究通过分析胃癌患者的临床资料、对比胃癌组织和癌旁组织ICMT的表达差异,评价ICMT对胃癌疾病进程的影响,为今后胃癌的防治探索新的方向。

1　对象与方法

1.1　*研究对象*　回顾性收集兰州大学第二医院2022年5月至9月确诊为胃癌并行手术治疗的60例患者的临床资料及手术标本。所有纳入的患者均符合胃癌的诊断标准,依据2016年美国肿瘤联合会(American Joint Committee on Cancer, AJCC)联合国际抗癌联盟(Union for International Cancer Control, UICC)颁布的第8版TNM分期系统[10],对胃癌患者进行临床病理分期。同时排除合并有其他恶性肿瘤、存在精神疾病或情绪不稳定而无法配合的患者。所有纳入的患者术前均未接受放疗或化疗,且临床资料完整,均已签署知情同意书。研究已通过兰州大学第二医院医学伦理委员会审查(2023A-068)。

1.2　*标本取材*　在胃癌标本离体后的15 min内,从胃癌组织无出血和坏死的部位取适当大小的癌组织,再从胃癌标本切缘处(距胃癌组织3~5 cm)取适当大小的癌旁正常组织,分别放入预先编号的冻存管中,迅速置于-80 ℃冰箱中保存,备用。免疫组织化学染色(immunohistochemistry, IHC)所用切片借自兰州大学第二医院病理科的手术标本库。

1.3　*材料与方法*

1.3.1　IHC染色实验　将石蜡切片依次进行脱脂、脱蜡复水、抗原修复后,将切片放入10%的过氧化氢溶液中,室温避光孵育20 min。滴加5%牛血清白蛋白,室温封闭1 h。轻轻甩去封闭液,滴加ICMT抗体(GeneTex, GTX129471),4 ℃孵育过夜。次日用磷酸盐缓冲盐水清洗后滴加山羊抗兔IgG二抗(Abbkine, A21020),室温孵育50 min。再次清洗后滴加二氨基联苯胺显色液(Beyotime, P0203)。Harris苏木素复染,脱水封片。Tissue FAXS Plus全景组织细胞定量系统(TissueGnostics Asia Pacific)观察并采集图像。每个样品随机取5个视野,利用Image-Pro Plus软件分析图像并计算每个视野中阳性颗粒(棕黄色)的平均光密度值,最后取平均值以反映目的蛋白的表达强度。

1.3.2　聚合酶链式反应(polymerase chain reaction, PCR)　实验提取组织总RNA:迅速取出存放于-80 ℃冰箱的胃癌及癌旁组织,放入预先加入适量RNA提取液的离心管中,组织研磨器于冰上将组织研碎。随后加入三氯甲烷,剧烈振荡15 s,冰上裂解30 min,4 ℃、离心15 min。吸取离心管上层的清亮液体,将其移至另一离心管中并加入等体积的异丙醇,摇晃混匀,静置10 min,再次离心10 min。向留存于管底的RNA沉淀中加入75%的乙醇。再次离心5 min,

重复此步操作 1~2 次。倒掉离心管内乙醇并倒置管体，自然晾干后在离心管内加入 20~40 μL 焦碳酸二乙酯水（Biosharp，143198）；利用微量核酸检测仪（Thermo Scientific，Nanodrop2000）检测所提取 RNA 的纯度及浓度。

RNA 逆转录：依据 RNA 反转录试剂盒（SparkJade，AG0304-B）的操作说明，全程在冰上操作配制反应液，并加入至 RNA 样品中置于普通 PCR 仪（BioGener，GE4852T）。随后在其中加入 10 μL SPARK script Ⅱ RT Plus Master Mix，继续放入普通 PCR 反应仪中进行反应，最终反转录得到 cDNA。实验独立地重复进行三次。

cDNA 扩增荧光定量：参考既往研究报道[11]，设计本实验所用的引物序列，分别是 ICMT 基因的正向引物：5′-CGC TTG GTT TCG GCA TCC TTC T-3′；反向引物：5′-CGG AAG AAT CGC CAC ACT GTC A-3′；内参基因 GAPDH 的引物序列为正向引物：5′-TGC ACC ACC AAC TGC TTA GC-3′；反向引物：5′-AGC TCA GGG ATG ACC TTG CC-3′。在扩增管中按 cDNA 扩增试剂盒（SparkJade，AH0104-B）的反应体系加入各试剂和上述引物，离心混匀后置于实时荧光定量 PCR 仪（QIAGEN，Rotor-Gene Q）中进行扩增荧光定量反应。实验独立地重复进行三次。

采用 $2^{-\Delta\Delta Ct}$ 法计算出胃癌组织和癌旁组织目的基因 ICMT mRNA 的相对表达量。与癌旁组织组相比，ICMT mRNA 的 $2^{-\Delta\Delta Ct}$ 值>1 者为高表达，<1 者为低表达。

1.4　统计学方法　采用 GraphPad Prism 8 软件进行实验结果分析并作图。计数资料以例（%）表述，用 χ^2 检验分析 ICMT 与胃癌患者临床特征之间的关联性。P<0.05 为差异有统计学意义。

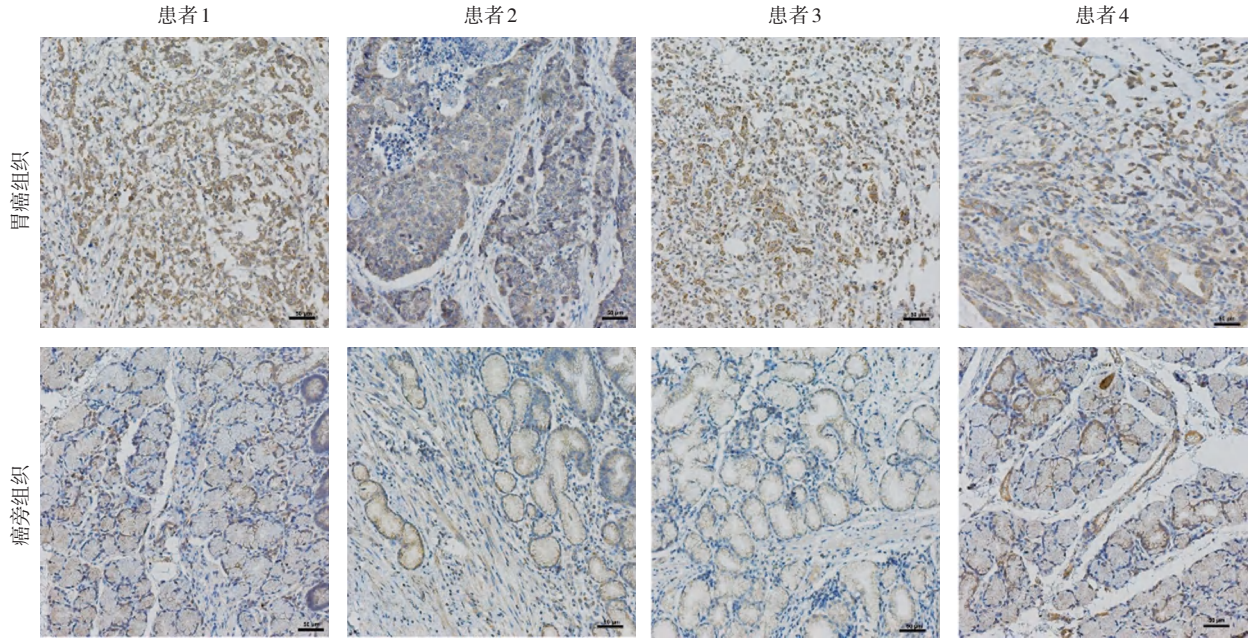
2　结　果

2.1　ICMT 蛋白在两种组织中的表达　利用 IHC 技术，观察胃癌患者癌组织与癌旁组织 ICMT 蛋白的表达情况。如图 1 所示，胃癌组织结构紊乱、细胞排列杂乱、极向丧失，胞浆中 ICMT 蛋白的阳性颗粒数显著高于癌旁组织。

2.2　ICMT mRNA 在两种组织中的表达　利用 PCR 技术，对比 60 例胃癌患者的胃癌组织及癌旁组织 ICMT 的 mRNA 相对表达水平，如图 2 所示，ICMT 在胃癌组织的表达水平显著高于癌旁组织（P<0.05）。

2.3　ICMT mRNA 与胃癌患者临床特征的关联性分析　胃癌组织中 ICMT 高表达 34 例（56.67%）、低表达 26 例（43.33%）；癌旁组织中 ICMT 高表达 22 例（36.67%）、低表达 38 例（63.33%），胃癌组织中 ICMT 高表达比例显著高于癌旁组织，差异有统计学意义（χ^2 = 4.821，P = 0.028）。

分析胃癌患者的临床特征发现，（1）部位：ICMT 在不同部位胃癌中的表达差异有统计学意义（χ^2=



注：从左向右依次为患者 1、2、3、4 的胃癌组织及其癌旁组织 IHC 染色（比例尺=50 μm）。

图 1　胃癌组织及和癌旁组织 ICMT 蛋白的表达

Fig.1　The expression level of ICMT protein in gastric tumor tissue and adjacent tissue

7.161，*P*=0.028），ICMT 高表达比例胃体癌最高，贲门癌其次，胃窦胃癌最低。（2）组织学特征：ICMT 的表达水平与 Lauren 分型具有显著关联性（χ^2 = 13.153，*P*=0.001），ICMT 高表达比例混合型胃癌最高，肠型其次，弥漫型最低。（3）分化程度：低分化癌组织的 ICMT 水平显著高于中高分化组（χ^2 = 7.625，*P* = 0.006）。（4）TNM 分期及转移、侵犯：依据 T 分期（肿瘤的原发病灶情况及浸润），处于 T3+T4 期的胃癌组织中 ICMT 显著高表达（χ^2 = 5.740，*P* = 0.017）；而 ICMT 的表达差异与 N 分期（淋巴结受累情况）、M 分期（肿瘤的远处转移）、血管侵犯及神经侵犯等均无显著关联性（*P*>0.05）。（5）p53 蛋白表达：p53 突变型患者 ICMT 的高表达比率较 p53 野生型者显著增高（χ^2 = 5.831，*P*=0.016）。见表 1。

表 1　胃癌患者 ICMT mRNA 表达水平与临床特征的相关性　［例（%）］

Tab.1　Correlation between the expression levels of ICMT mRNA and clinical characteristics in gastric cancer patients　［case (%)］

临床特征	例数	ICMT 高表达（*n*=34）	ICMT 低表达（*n*=26）	χ^2值	*P*值
性别					
男	41	24(58.54)	17(41.46)	0.184	0.668
女	19	10(52.63)	9(47.37)		
年龄					
<60 岁	24	13(54.17)	11(45.83)	0.102	0.750
≥60 岁	36	21(58.33)	15(41.67)		
肿瘤大小					
< 5 cm	22	10(45.45)	12(54.55)	1.778	0.182
≥ 5 cm	38	24(63.16)	14(36.84)		
病变部位					
胃上部（贲门）	19	12(63.16)	7(36.84)		
胃中部（胃体）	15	12(80.00)	3(20.00)	7.161	0.028
胃下部（胃窦）	26	10(38.46)	16(61.54)		
浸润深度					
黏膜下层-肌层	19	12(63.16)	7(36.84)	0.477	0.450
浆膜-全层	41	22(53.66)	19(46.34)		
胃癌的组织类型					
腺癌	58	33(56.90)	25(43.10)	0.037	0.194
印戒细胞癌	2	1(50.00)	1(50.00)		
Lauren 分型					
弥漫型	17	4(23.53)	13(76.47)		
肠型	18	10(55.56)	8(44.44)	13.153	0.001
混合型	25	20(80.00)	5(20.00)		
分化程度					
低分化	47	31(65.96)	16(34.04)	7.625	0.006
中高分化	13	3(23.08)	10(76.92)		
T 分期					
T1+T2	16	5(31.25)	11(68.75)	5.740	0.017
T3+T4	44	29(65.91)	15(34.09)		
N 分期					
N0	17	10(58.82)	7(41.18)	0.045	0.832
N1~3	43	24(55.81)	19(44.19)		
M 分期					
M0	56	33(58.93)	23(41.07)	1.750	0.186
M1	4	1(25.00)	3(75.00)		
血管侵犯					
是	51	27(52.94)	24(47.06)	1.922	0.166
否	9	7(77.78)	2(22.22)		
神经侵犯					
是	51	27(52.94)	24(47.06)	1.922	0.166
否	9	7(77.78)	2(22.22)		
癌组织的 p53 表达					
野生型	22	8(36.36)	14(63.64)	5.831	0.016
突变型	38	26(68.42)	12(31.58)		

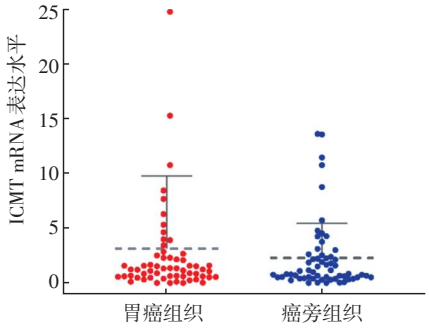


图 2　胃癌组织及和癌旁组织 ICMT mRNA 的表达水平

Fig.2　The expression levels of ICMT mRNA in gastric tumor tissue and adjacent tissue

3　讨　论

ICMT 是 Ras 信号通路的关键酶，其所参与的特定甲基化事件是 Ras 蛋白翻译后修饰的最后一步。既往研究证实，抑制 ICMT 可改善恶性肿瘤的表型。癌症基因组图谱数据库的数据显示，胃癌中 *Ras* 突变最常见的类型是 *K-Ras* 突变[12]。Peng 等[13]报道使用 Nested 和 COLD-PCR 方法检测了 126 份胃癌患者的组织和 9 份血浆样本，其中 *K-Ras* 突变发生率为 6.67%。另有研究表明，*K-Ras* 突变可能参与分化型胃癌的早期癌变[14]。本研究显示，ICMT 的 mRNA 及蛋白表达水平在胃癌组织中均显著高于癌旁正常组织，ICMT 高表达比例在低分化胃癌组织中显著高于中高分化；在 T1+T2 期显著低于 T3+T4 期。上述研究结果均提示胃癌患者群体中 ICMT 表达上调是一个分子特征，且与肿瘤的恶性程度及疾病进展密切相关。结合 ICMT 的分子特征及作用特点，认为 ICMT 基因可能是与胃癌相关的一种癌基因，可通过调节其下游的效应分子促进胃癌的发生和进展。

马婷等[15]回顾分析了我国 6 446 例胃癌患者的内镜下表现，其中发生在贲门胃底部的胃癌比例高达

50.5%。Yang 等[16]开展的大样本病例对照研究显示，78.5%的非贲门癌和62.1%的贲门癌病例可归因于幽门螺杆菌(*Helicobacter pylori*, Hp)感染。Hp 主要定植于胃窦部，也可随胃食管反流到达贲门而致病。Hp 产生的多种毒力因子能够激活包括 Ras 等多条信号通路[8,17]，损伤黏膜引发炎症，甚至癌变。本研究纳入的贲门癌虽然只有 19 例，但其中的 12 例呈现 ICMT 蛋白的高水平，提示 ICMT 可能与贲门癌的发病有关。

抑癌基因 *p53* 突变是人类恶性肿瘤中常见的体细胞事件之一，突变体可通过获得新的活性而参与肿瘤的发生和发展，涉及不同的机制[18-19]。突变型 *p53* 能够改变甲羟戊酸途径的基因表达[20]，而 ICMT 是该途径重要的代谢产物，是负责编码甲基转移酶的重要基因，也是参与蛋白质异戊二烯化最后一步的关键酶。*p53* 的状态影响 ICMT mRNA 和蛋白质的水平，即突变型 *p53* 促进 ICMT 的表达[18]；野生型 *p53* 则作用相反，原因在于野生型 p53 抑制了 ICMT 启动子-209 至-14 的区域。一旦 *p53* 发生突变，则上述抑制效应会被抵消，进而协同 ICMT 的表达[21]，并在不同水平上促进肿瘤发生中关键蛋白(如 Ras 等)的异戊二烯化，强化肿瘤的恶性生物学行为。该研究还通过比较 *p53* 状态与乳腺癌及肺癌中 ICMT 表达之间的相关性，进一步证实了上述结论[21]。本研究发现，具有 *p53* 突变型的胃癌其高表达 ICMT 的占比更高，与上述研究结果相似。机体抑癌基因与原癌基因的平衡被破坏是肿瘤发生的关键。抑癌基因 *p53* 与癌基因 *Ras* 被认为是细胞命运的决定者，二者之间存在复杂的相互作用，表现为相互调控或协同调节某些关键的肿瘤相关基因[19]。ICMT 是 Ras 激活的关键酶之一，因此，笔者推测抑癌基因 *p53* 的突变和 Ras 的过度激活可能是 ICMT 高表达的分子基础，也可能是二者之间相互协同作用的结果，但具体作用关系尚需进一步研究证实。

综上所述，本研究分析了 ICMT 蛋白表达水平与胃癌患者临床及病理特征的关联性，发现 ICMT 在胃癌患者中高表达，且表达差异与胃癌的病变部位、分化程度、病程阶段存在关联，*p53* 突变可能是胃癌组织 ICMT 高表达的病理基础，这可能为今后胃癌的防治探索提供新的视角。

利益冲突　无

参考文献

[1] Sabt A, Tawfik HO, Khaleel EF, et al. An overview of recent advancements in small molecules suppression of oncogenic signaling of K-RAS: an updated review[J]. Mol Divers, 2024, 28(6): 4581-4608.
[2] Chen K, Zhang YL, Qian L, et al. Emerging strategies to target RAS signaling in human cancer therapy[J]. J Hematol Oncol, 2021, 14(1): 116.
[3] Luo X, Xie FM, Qin GQ, et al. circICMT upregulates and suppresses the malignant behavior of bladder cancer[J]. Transl Oncol, 2025, 52: 102262.
[4] Masago K, Kuroda H, Sasaki E, et al. Novel gene fusions in human oropharyngeal carcinoma[J]. Cancer Genet, 2024, 286/287: 29-34.
[5] Mouhcine M, Kadil Y, Segmani I, et al. In silicoexploration of a novel ICMT inhibitor with more solubility than cysmethynil against membrane localization of *KRAS* mutant in colorectal cancer[J]. Curr Comput Aided Drug Des, 2024, 20(7): 1055-1069.
[6] Zhao JX, Zhao ZQ, Hou WT, et al. Quantitative proteomics explore the potential targets and action mechanisms of hydroxychloroquine[J]. Molecules, 2022, 27(16): 5175.
[7] Sundar R, Nakayama I, Markar SR, et al. Gastric cancer[J]. Lancet, 2025, 405(10494): 2087-2102.
[8] Morgos DT, Stefani C, Miricescu D, et al. Targeting PI3K/AKT/mTOR and MAPK signaling pathways in gastric cancer[J]. Int J Mol Sci, 2024, 25(3): 1848.
[9] 黄荧, 魏嘉, 刘宝瑞. *K-ras* 突变在胃癌预后及靶向治疗中的研究进展[J]. 中华肿瘤杂志, 2016, 38(2): 81-85.
[10] Amin MB, Edge SB, Greene FL. AJCC Cancer Staging Manual[M]. 8th ed. New York: Springer, 2016: 203-220.
[11] Li YJ, Gao XR, Yang CC, et al. CircRNA hsa_circ_0018289 exerts an oncogenic role in cervical cancer progression through miR-1294/ICMT axis[J]. J Clin Lab Anal, 2022, 36(5): e24348.
[12] Chen SJ, Li FY, Xu D, et al. The function of *RAS* mutation in cancer and advances in its drug research[J]. Curr Pharm Des, 2019, 25(10): 1105-1114.
[13] Peng NQ, Zhao XT. Comparison of *K-ras* mutations in lung, colorectal and gastric cancer[J]. Oncol Lett, 2014, 8(2): 561-565.
[14] Hiyama T, Haruma K, Kitadai Y, et al. *K-ras* mutation in *Helicobacter pylori*-associated chronic gastritis in patients with and without gastric cancer[J]. Int J Cancer, 2002, 97(5): 562-566.
[15] 马婷, 隋玥, 卢俊会, 等. 6 446 例胃癌内镜下病灶部位特点回顾性分析[J]. 现代消化及介入诊疗, 2022, 27(6): 702-706.
[16] Yang L, Kartsonaki C, Yao P, et al. The relative and attributable risks of cardia and non-cardia gastric cancer associated with *Helicobacter pylori* infection in China: a case-cohort study[J]. Lancet Public Health, 2021, 6(12): e888-e896.
[17] Alipour M. Molecular mechanism of *Helicobacter pylori*-induced gastric cancer[J]. J Gastrointest Cancer, 2021, 52(1): 23-30.
[18] Borini Etichetti CM, Arel Zalazar E, Cocordano N, et al. Beyond the mevalonate pathway: control of post-prenylation processing by mutant *p53*[J]. Front Oncol, 2020, 10: 595034.
[19] 袁敏, 朱心怡, 郑佳谊, 等. 胃癌组织中 EB 病毒感染与 *p53* 蛋白表达及临床病理特征[J]. 中华转移性肿瘤杂志, 2024, 7(2): 179-183.
[20] Freed-Pastor WA, Mizuno H, Zhao X, et al. Mutant *p53* disrupts mammary tissue architecture via the mevalonate pathway[J]. Cell, 2012, 148(1/2): 244-258.
[21] Borini Etichetti C, Di Benedetto C, Rossi C, et al. Isoprenylcysteine carboxy methyltransferase (ICMT) is associated with tumor aggressiveness and its expression is controlled by the *p53* tumor suppressor[J]. J Biol Chem, 2019, 294(13): 5060-5073.

收稿日期：2025-04-16　**修回日期**：2025-06-26　**编辑**：石嘉莹