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Original Article

Clinical significance of MED12 expression in colorectal cancer

Yoshifumi Shimada[1], Yosuke Tajima[1], Hitoshi Kameyama[1], Ryoma Yagi[1], Takuma Okamura[1], Yuki Hirose[1], Jun Sakata[1], Takashi Kobayashi[1], Yasunobu Matsuda[2], Yoichi Ajioka[3], Shin-ichi Kosugi[4], Toshifumi Wakai[1]

[1]Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; [2]Department of Medical Technology, Niigata University Graduate School of Health Sciences, Niigata, Japan; [3]Division of Molecular and Diagnostic Pathology, Niigata University Graduate School of Medical and Dental Sciences, Niigata, Japan; [4]Department of Digestive and General Surgery, Uonuma Institute of Community Medicine, Niigata University Medical and Dental Hospital, Niigata, Japan

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Abstract: MED12 is a transcriptional mediator complex subunit, which negatively regulates the transforming growth factor β (TGF-β) pathway. The TGF-β pathway plays a major role in the induction of epithelial-mesenchymal transition (EMT). MED12 loss induces activation of the TGF-β pathway, resulting in EMT and drug resistance to epidermal growth factor receptor (EGFR)-targeted therapy. We aimed to investigate the clinical significance of MED12 loss detected by immunohistochemistry in patients with colorectal cancer (CRC). A total of 100 patients diagnosed with stage I-IV CRC were enrolled in this retrospective study. MED12 expression was evaluated immunohistochemically, and classified as either positive (≥20%) or negative (<20%) with regard to the percentage of immunoreactive cells. The relationships between MED12 loss and clinicopathological characteristics and RAS mutation status were analyzed. Overall, 79 and 21 patients were classified as MED12 positive and MED12 negative, respectively. MED12 negativity was significantly associated with tumor budding ($P = 0.034$), N category ($P = 0.010$), and M category ($P = 0.031$). Among stage IV CRC patients, 18 of 31 patients had the RAS wild-type gene; 6 of these patients were MED12 negative, and were considered to have the potential for resistance to EGFR-targeted therapy despite the presence of the wild-type gene. In conclusion, MED12 loss is associated with tumor budding, nodal metastasis, and distant metastasis in patients with CRC, suggesting that MED12 loss induces activation of the TGF-β pathway resulting in EMT. Future treatment strategies focusing on patients MED12 loss may improve the prognosis of patients with CRC.

Keywords: MED12, EMT, TGF-β, RAS, immunohistochemistry, colorectal cancer

Introduction

The second most common malignancy in developed countries is colorectal cancer (CRC) [1]. TNM stage is mainly determined according to the absence/presence of nodal and distant metastases, and the prognosis of CRC patients mainly depends on the absence/presence of these metastases [2]. Patients with nodal metastasis assigned to stage III according to the TNM classification have a 5-year overall survival (OS) rate of approximately 30%-70% [2]; patients with distant metastasis assigned to stage IV have a 5-year OS rate of approximately 5% [2]. These OS rates are much worse than those of stage I/II patients; hence, treatment for patients with metastasis is the key to improving the prognosis of CRC patients.

Epithelial-mesenchymal transition (EMT) is thought to be one of the main mechanisms of metastasis in various cancers [3, 4]. EMT is a highly conserved program that converts immotile, polarized epithelial cells into motile mesenchymal cells. First noted in several critical stages of embryonic development, EMT has also been linked to promotion of carcinoma invasion and metastasis [4]. It is known that several signaling pathways and transcription factors regulate the EMT program in carcinoma cells. Among them, the transforming growth factor β (TGF-β) pathway plays a major role in the induction of EMT [3, 4], which represses the expression of E-cadherin and leads to a subsequent loss of cell-to-cell adhesion [5, 6].

In human cancer cell lines and mouse tumor models, activation of the TGF-β pathway allows

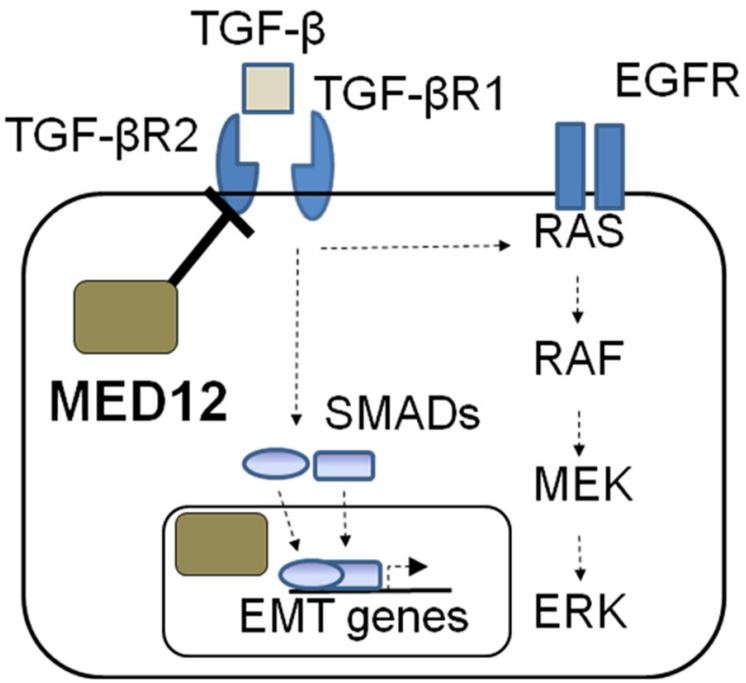

Figure 1. Function of MED12 in the TGF-β and MAPK pathways. MED12 exists in the nucleus and cytoplasm. Cytoplasmic MED12 negatively regulates TGF-βR2 through physical interaction.

these cells to invade the extracellular matrix in culture and proliferate into various systems in mice [7]. In the canonical TGF-β pathway, type 2 TGF-β receptor (TGF-βR2) activates TGF-βR1. This then phosphorylates two transcription factors, SMAD2 and SMAD3, which translocate to the nucleus and regulate TGF-β-targeted gene expression, inducing EMT through the phosphorylation of β-catenin, leading to upregulation of S-nail, vimentin, and N-cadherin [9]. On the other hand, a collateral TGF-β pathway also exists, which transduces to the mitogen-activated protein kinase (MAPK) pathway that is associated with cancer cell proliferation [9].

Recently, a mechanism of drug resistance induced by activating the TGF-β pathway has been highlighted: the transcriptional mediator complex subunit 12 (MED12) negatively regulates TGF-βR2 through physical interaction (**Figure 1**), and MED12 loss has been shown to induce activation of the TGF-β pathway, resulting in drug resistance in colon and lung cancer [9]. MED12 loss induces activation of both the canonical and collateral TGF-β pathways. In the canonical TGF-β pathway, MED12 loss induces an EMT-like phenotype of cancer cells, which is associated with chemotherapy resistance. In the collateral TGF-β pathway, MED12 loss activates the MAPK pathway, causing resistance to epidermal growth factor receptor (EGFR) inhibition [10, 11].

Although previous studies have demonstrated that MED12 loss induces the activation of the TGF-β pathway and EMT, no studies have shown the association between MED12 loss and clinicopathological characteristics including RAS mutation status in CRC patients. In the present study, we analyzed MED12 expression by immunohistochemistry (IHC) to investigate the association between MED12 expression and EMT morphology such as tumor budding [12], and determined the clinical significance of MED12 loss detected by IHC in CRC.

Materials and methods

Patients

A total of 100 patients diagnosed with stage I-IV CRC according to AJCC 7th edition [2] who underwent curative surgery between 2013 and 2015 at Niigata University Medical and Dental Hospital or Niigata Cancer Center Hospital were enrolled in the study (**Table 1**). Patients with familial adenoma polyposis, inflammatory bowel disease, or synchronous multiple CRCs were excluded. No patients received neoadjuvant radiation. This retrospective study was performed in accordance with the Declaration of Helsinki, and the Ethics Committee of the School of Medicine, Niigata University approved the study protocol.

Pathological evaluation of MED12 expression

Mesenteric lymph nodes were dissected immediately following surgery by excising the mesenteric fatty tissue prior to histological examination. The bowel was immediately fixed in 10% buffered formalin and the entire tumor was cut into stepwise sections and embedded in paraffin. Each section was examined with hematoxylin and eosin (HE) staining, and reviewed archives to select one a cross-section for each case that had a good degree of invasion.

Table 1. Clinicopathological characteristics of 100 patients

Variable	
Age (years)[a]	65 (30-94)
Sex	
Male : Female	55:45
Location	
Right sided : Left sided	27:73
Tumor size (mm)[a]	48 (9-125)
T category[b]	
T1 : T2 : T3 : T4	4:15:58:23
Histopathological grading	
G1 : G2 : G3	8:68:24
Tumor budding	
Low : High	93:7
Lymphatic invasion	
Absence : Presence	56:44
Venous invasion	
Absence : Presence	31:69
N category[b]	
N0 : N1 : N2	41:33:26
M category[b]	
Absence : Presence	69:31
Stage[b]	
I : II : III : IV	10:25:34:31
MED12 IHC	
Positive : Negative	79:21

[a]Data are expressed as median (range); [b]According to the AJCC Cancer Staging Manual [2]. Abbreviation: IHC (immunohistochemistry).

Corresponding paraffin blocks were re-cut into 4-mm thick slices, and 3 serial sections were assigned for HE staining, anti-MED12 staining, and as a negative control. Positive staining of MED12 in the nucleus and cytoplasm of cancer cells was regarded as immunoreactive and classified as either positive (≥20%) or negative (<20%) in terms of the percentage of immunoreactive cells among all cancer cells (**Figure 2**).

IHC for MED12 expression

Anti-MED12 antibody was purchased from Novus Biologicals (Littleton, CO). For IHC, sections were deparaffinized and rehydrated before being microwaving at 500 W for 21 minutes in 10 mM sodium citrate buffer (pH 6.0) to retrieve antigenic activity. Endogenous peroxidase activity was blocked by incubation with 0.3% hydrogen peroxide in methanol for 20 minutes. After blocking non-specific reactions with 10% normal goat serum, sections were incubated overnight at 4°C with MED12 rabbit monoclonal antibody (Epitomics; 1:100 dilution) as the primary antibody and then incubated at room temperature for 30 minutes with goat anti-rabbit IgG polymerized horseradish peroxidase-labelled secondary antibody (Epitomics). Diaminobenzidine was used as the chromogen and sections were counterstained with hematoxylin. Normal rabbit immunoglobulin was used instead of primary antibody in the negative control.

Diagnosis of tumor budding

Tumor budding was evaluated blinded to the clinical status, and defined as an isolated single cancer cell or a cluster of less than 5 cancer cells in the stroma in the invasive front area, according to the definition proposed by Ueno et al. [12]. One field in which tumor budding was most intensive was chosen, and budding counts were evaluated in this field using a × 20 objective lens. In the present study, budding counts of 10 or more were categorized as budding high (**Figure 3**).

Evaluation of RAS (KRAS and NRAS) mutation status

Detection of exons 2, 3, and 4RAS mutation was carried out on DNA extracted from manual microdissected tumor sections prepared from archival formalin-fixed paraffin-embedded samples using the RASKET KIT (Medical & Biological Laboratories, Nagoya, Japan) [13].

Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 22 (IBM Japan Inc., Tokyo, Japan). The relationships between MED12 expression and clinicopathological variables were analyzed using Fisher's exact test. *P* values less than 0.05 were considered statistically significant.

Results

MED12 expression and other clinicopathological characteristics

According to the diagnostic criteria of MED12 expression, 79 and 21 tumors were classified as MED12 positive and negative, respectively. MED12 loss was observed mainly at the tumor

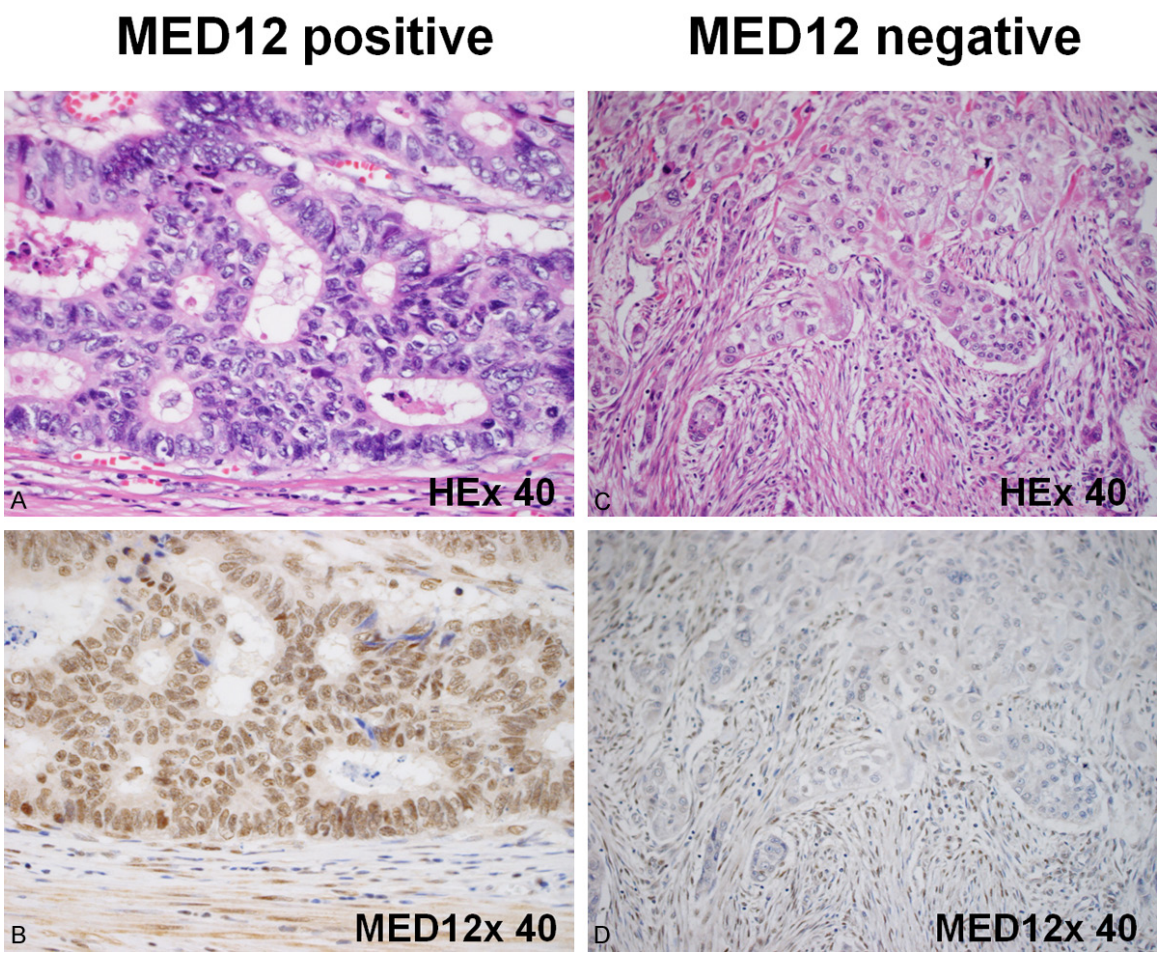

Figure 2. IHC for MED12 in CRC. A, B. In MED12 expression-positive cases, MED12 staining was detected in the nucleus and cytoplasm. C, D. Conversely, in MED12 expression-negative cases, MED12 staining was not detected. A, C. HE staining, × 40 objective lens. B, D. Anti-MED12 staining, × 40 objective lens.

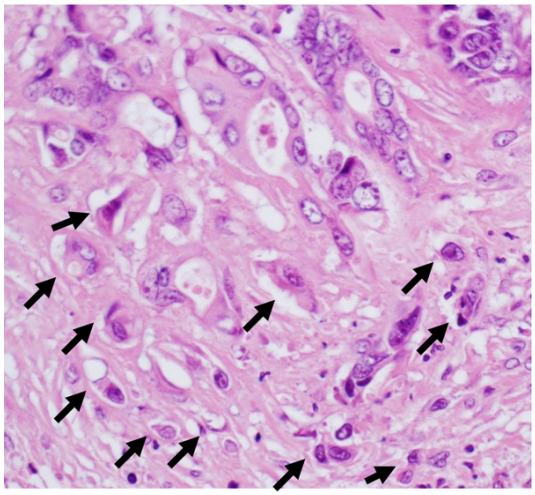

Figure 3. Tumor budding in CRC. Tumor budding was observed at the tumor invasive front. Arrows indicate tumor budding. HE staining, × 20 objective lens.

invasive front. However, in MED12-negative patients, MED12 loss was observed not only at the tumor invasive front but throughout the tumor. MED12 negativity was significantly associated with tumor budding (P = 0.034), N category (P = 0.010), and M category (P = 0.031), whereas there were no significant associations between MED12 expression and other clinicopathological characteristics (**Table 2**).

Correlation between MED12 loss and sites of distant metastasis

Liver, lung, and peritoneal metastases and extra-regional lymph nodes were observed in 21, 7, 6, and 4 patients, respectively. There were no significant associations between MED12 loss and any sites of distant metastasis.

Table 2. Association between MED12 immunohistochemistry and other clinicopathological characteristics

Variable	MED12 IHC		*P* value
	Positive (*n* = 79)	Negative (*n* = 21)	
Age (years)			
<65	35	11	0.624
≥65	44	10	
Sex			
Male	42	13	0.623
Female	37	8	
Location			
Right-sided	20	7	0.581
Left-sided	59	14	
Tumor size (mm)			
<50	41	11	0.999
≥50	38	10	
T category[a]			
T1, 2	15	4	0.999
T3, 4	64	17	
Histopathological grading			
G1, 2	59	17	0.775
G3	20	4	
Tumor budding			
Low	76	17	0.034
High	3	4	
Lymphatic invasion			
Absence	43	13	0.581
Presence	36	8	
Venous invasion			
Absence	23	8	0.437
Presence	56	13	
N category[a]			
N0	32	9	0.010
N1	31	2	
N2	16	10	
M category[a]			
M0	59	10	0.031
M1	20	11	

[a]According to the AJCC Cancer Staging Manual [2]. Abbreviation: IHC (immunohistochemistry).

MED12 expression and mutation in the MAPK pathway in stage IV patients

Among stage IV patients, KRAS and NRAS mutations were detected in 12 and 1 patients, respectively. MED12 loss was observed in 11 patients. Thirty-one patients with stage IV CRC were divided into 4 groups according to RAS mutation status and MED12 expression (**Figure 4**).

Discussion

In the present study, there were two main findings related to MED12 expression in CRC. First, MED12 expression detected by IHC was lost in 21 (21%) patients with CRC. Second, MED12 loss was significantly associated with tumor budding, lymph node metastasis, and distant metastasis. These results imply that MED12 loss is a possible mechanism of invasion and metastasis in CRC, and this phenomenon may be explained by the theory of EMT derived from TGF-β pathway activation induced by MED12 loss.

MED12 is a mediator subunit that is necessary for successful gene transcription in all eukaryotic cells [14, 15]. The mediator complex, consisting of at least 26 subunits, regulates RNA polymerase II recruitment and stabilization, chromatin remodeling, transcription factor recognition, and transcription elongation [14, 15]. Huang et al. revealed an unexpected role for MED12 in drug resistance through activation of the TGF-β pathway, and proposed a new combination cancer treatment in which inhibition of the TGF-β pathway resensitizes cells to therapeutic drugs [9]. However, there have been few reports regarding the role of MED12 in patients with CRC, and the association between MED12 expression and clinicopathological features has not been elucidated in these patients. To our knowledge, this is the first report of the correlation between MED12 loss and tumor budding, nodal metastasis, and distant metastasis in patients with CRC.

Tumor budding is a typical morphological feature of the malignant cancer phenotype, which predicts lymph node metastasis, distant metastasis, and poor disease-free survival in CRC [16-18]. Recent evidence supports the notion that tumor budding represents the morphological correlate of cancer cells having undergone EMT, which is an important mechanism for the progression of epithelial cancers [16]. Although there is a lack of supporting evidence originating from human tissues, there are some data from a limited number of studies showing that drug resistance is associated with EMT in CRC [19, 20]. In the present study, we

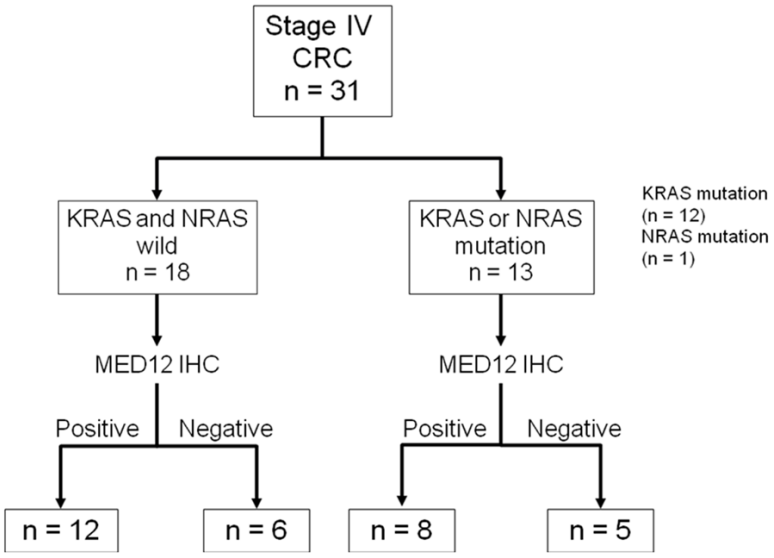

Figure 4. RAS mutation status and MED12 expression detected with IHC in patients with stage IV CRC. Eighteen of 31 patients had the KRAS and NRAS wild-type genes, and these patients were considered to be candidates for EGFR-targeted therapy. However, 6 of 18 patients showed loss of MED12 expression, and these patients were considered to have the potential for drug resistance to EGFR-targeted therapy.

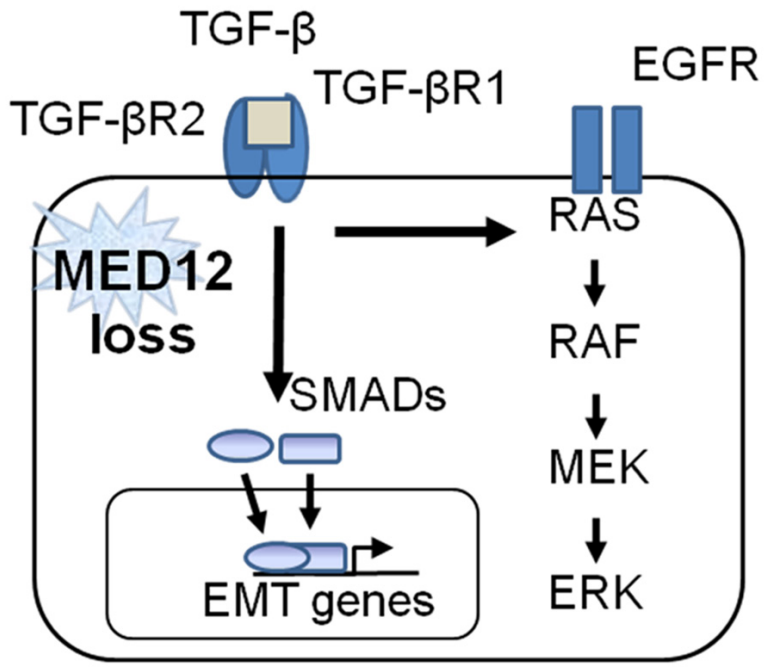

Figure 5. MED12 loss in the TGF-β and MAPK pathways. MED12 loss induces activation of the canonical TGF-β pathway, as well as the collateral TGF-β pathway which activates the MAPK pathway.

revealed the correlation between MED12 and tumor budding, indicating that tumor budding may be the morphological phenotype of MED12 loss and subsequent TGF-β activation. Hence, we consider that inhibition of the TGF-β pathway for MED12 loss is a potential therapeutic strategy for patients with tumor budding with malignant potential [9, 21, 22].

Gene mutations in the MAPK pathway, such as KRAS, NRAS, and BRAF, are important benchmarks to determine treatment strategies for patients with metastatic CRC. National Comprehensive Cancer Network guidelines state that tumor tissue should be genotyped for KRAS, NRAS, and BRAF mutations for all patients with metastatic CRC. Patients with any known KRAS or NRAS mutation should not be treated with EGFR-targeted therapy such as cetuximab and panitumumab [23-25]. In the present study, we observed that 18 of 31 (58%) patients with stage IV disease were RAS wild-type; such patients are thought to be candidates for EGFR-targeted therapy. According to MED12 expression, the 18 RAS wild-type patients were further divided into two groups: 12 (67%) were MED12 positive and 6 (33%) were MED12 negative (**Figure 4**). We speculate that MED12 loss in the latter group causes stimulation of the collateral TGF-β pathway, which stimulates the MAPK pathway and causes resistance to EGFR-targeted therapy despite the presence of RAS wild-type (**Figure 5**).

This study has three potential limitations. First, this was a retrospective study performed at 2 institutions, and included only a small number of patients.

Second, we could not demonstrate the association between MED12 loss and survival outcome because of the short observation period. Third, we could not demonstrate a correlation between MED12 loss and resistance to EGFR-targeted therapy. Future studies should focus on patients with unresectable CRC to determine whether MED12 loss is correlated with resistance to EGFR-targeted therapy, and to establish treatment strategies for patients with MED12 loss.

In conclusion, MED12 loss is associated with tumor budding, nodal metastasis, and distant metastasis in CRC patients, suggesting that MED12 loss induces activation of the TGF-β pathway resulting in EMT. Future treatment strategies focusing on patients MED12 loss may improve the prognosis of patients with CRC.

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Disclosure of conflict of interest

None.

Address correspondence to: Yoshifumi Shimada, Division of Digestive and General Surgery, Niigata University Graduate School of Medical and Dental Sciences, 1-757 Asahimachi-Dori, Chuo-Ku, Niigata 951-8510, Japan. Tel: +81-25-227-2228; Fax: +81-25-227-0779; E-mail: shimaday@med.niigata-u.ac.jp

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