CASE REPORT

A Case of Colonic Micropapillary Carcinoma with a High Frequency of Apoptosis

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Abstract
Colorectal micropapillary carcinoma (MPC) exhibits aggressive biological characteristics, with empty spaces and reversed polarity, similar to the poorly differentiated clusters (PDCs) formed from detached cancer cells. Epithelial–mesenchymal transition, which is involved in the cancer cell acquisition of apoptosis resistance, is closely linked with histological findings of MPC, PDCs, and tumor buds (TBs), with MPC and TBs considered as apoptosis-resistant features. However, we encountered a case of colonic MPC with frequent apoptosis. We examined the case using immunohistochemistry. In many of the tumor glands (TGs) of the MPC, empty spaces and tumor cell detachment toward the gland interior were observed. Moreover, TG ruptures were scattered, with PDCs adjacent to them. Apoptosis occurred mainly at the TG and PDC peripheries in the middle and deep tumor layers, and transforming growth factor beta 1 (TGF-β1) positivity was evident in those tumor cells. Cells positive for apoptosis-related M30 were distributed mainly in the deep layer with a significant PDC and TB presence. However, apoptosis and M30 positivity were low in the TBs. Non-tumorous bud components, especially those in the deep layer, had poor ability to promptly acquire apoptosis resistance. No nuclear β-catenin positivity was found in any of the tumor cells. Apoptosis has the potential to reciprocally produce MPC, PDCs, and TBs, with TGF-β1 involvement.

Keywords Apoptosis · Colorectal cancer · Immunohistochemistry · Micropapillary carcinoma · Tumor budding

Introduction
Micropapillary carcinoma (MPC) of the colon, an uncommon subtype of colorectal cancer (CRC), generally coexists with components of conventional adenocarcinoma (AC) [1–4]. MPC of the colon or rectum, like that of various other organs, is known to display aggressive biological characteristics, such as lymphovascular invasion and node metastasis, and generally has a poor prognosis [2–5]. Additionally, regardless of the primary organ, MPC is characterized by neoplastic cell clusters that are located in empty spaces, resemble dilated lymphatic vessels, and show reversed polarity (i.e., inside-out structures) [1–5]. In CRC, poorly differentiated clusters (PDCs) are a tumor feature that has much in common with the clinicopathological and morphological characteristics of MPC [6–8], and the two structures may overlap pathogenetically [6–8]. PDCs, which are formed from cancer cells that have detached from a main tumor body, are defined as clusters of five or more neoplastic cells without glandular formation [8, 9]. A similar feature consisting of less than five neoplastic cells is the tumor bud (TB) [10], which represents a morphological continuum with the PDC [7–10] and also exhibits similar characteristics as colorectal MPC and PDCs (MPC/PDCs) [7, 8]. In fact, because of their clinicopathological and morphological similarities, colorectal MPC/PDCs and TBs are considered to be sequential growth patterns that branch from common biological processes [7], with PDC formation being regarded as the step prior to tumor budding [11].

Colorectal MPC/PDCs and TBs are considered to be closely linked with epithelial–mesenchymal transition (EMT) [4, 6, 7, 12–14]. In general, cancer cells that have undergone EMT acquire resistance to apoptosis have a higher invasive...
capacity and exhibit aggressive biological behavior [15, 16]. This observation is supported by reports that the TBs of CRC display apoptosis resistance, unlike the conventional AC components [17, 18]. Moreover, micropapillary structures have been shown to be one of the putative apoptosis-resistant subpopulations in CRC [19, 20]. Studies have also shown that KRAS mutations are related to the development of colorectal MPC/PDCs and TBs and are involved in the acquisition of apoptosis resistance by cancer cells [4, 18, 21, 22]. However, in contrast to these reports, we have encountered a case of colorectal MPC with frequent apoptosis. The tumor in this patient had many PDCs and also showed a high degree of tumor budding. Therefore, we examined the relationship between apoptosis and MPC/PDCs or TBs in the tumor with the unique histological findings. In this case, immunohistochemistry was performed using antibodies against M30 CytoDEATH (M30) (an apoptosis-related marker) [17, 19, 20, 23, 24], epithelial membrane antigen (EMA) (the gold-standard marker for luminal differentiation) [2, 3, 7, 24, 25], and transforming growth factor beta 1 (TGF-β1), E-cadherin, and β-catenin (the latter three all EMT-related markers) [6, 7, 12–14, 26–31]. This case highlights the fact that much about apoptosis and its relation to cancer pathology remains to be elucidated.

**Case History**

A 77-year-old woman presented with bloody stool that had persisted for the last 3 months. A colonoscopy revealed an ulcerated tumor in the sigmoid colon. She had a history of 15 years with diabetes mellitus and 7 years with chronic heart failure and atrial fibrillation, but she had neither a medical nor a family history of cancer. No metastases to the lymph nodes or other organs could be detected upon various imaging examinations, including computed tomography. With regard to tumor markers, the carcinoembryonic antigen (CEA) and cancer antigen 19–9 (CA-19–9) levels were within the normal range at 2.9 ng/ml (normal range 0–5 ng/ml) and less than 1.0 U/ml (normal range 0–37 U/ml), respectively. Because the colonoscopic biopsy revealed an AC, laparoscopic sigmoidectomy and lymph node dissection were performed. No analyses for mismatch repair (MMR) and the KRAS mutation status were performed. According to her medical history, she did not receive adjuvant therapy. Although the patient did not experience recurrence during the follow-up period of 16 months after surgery, she died suddenly of heart failure. An autopsy was not performed.

Three representative tissue sections, including both the tumor center and the tumor margin, were examined. TB or PDC scoring, with count correction for the objective lens, was performed as described elsewhere [9, 10]. Immunohistochemistry for podoplanin (D2-40) (a lymphatic vessel marker with a key role in cell invasiveness), M30, EMA, TGF-β1, E-cadherin, and β-catenin was carried out on serial tissue sections, using the Leica Bond-Max system (Leica Biosystems, Melbourne, Australia). The mouse anti-human D2-40 antibody (clone D2-40, ready to use, Nichirei Bioscience, Tokyo, Japan) was applied after subjecting the tissue sections to heat-induced antigen retrieval in a citrate-based solution (pH 6.0) for 20 min. Since this antibody reacts with the lymphatic endothelium, it is commonly used to distinguish between lymphatic vessels and empty spaces around tumor components [3, 6]. The conditions used for staining M30 and EMA are described elsewhere [24]. M30 detects apoptotic glandular-epithelial and AC cells through its recognition of a specific epitope of cytokeratin 18 [23] and is considered more specific and reliable than the TUNEL assay [23]. This antibody is often used for immunohistochemical studies of apoptosis resistance in AC [17, 19, 20]. EMA is used for the immunohistochemical demonstration of structures with reversed polarity [2, 3, 7, 24, 25]. The rabbit TGF-β1 polyclonal antibody (Proteintech Japan, Tokyo, Japan) [32] and mouse anti-human E-cadherin antibody (clone NCH-38, DAKO, Carpinteria, CA, USA), both used at a dilution ratio of 1:200, were applied to tissue sections that had been subjected to heat-induced antigen retrieval in an ethylenediaminetetraacetic acid-based solution (pH 9.0) for 20 min. The mouse anti-human β-catenin antibody (clone 17C2, Leica Microsystems, Newcastle Upon Tyne, Tyne and Wear, UK) was used at a 1:100 dilution ratio on tissue sections that had undergone heat-induced antigen retrieval in a citrate-based solution (pH 6.0) for 30 min. As a key event of EMT, the well-known dissolution of epithelial junctions is represented by the activation of signaling pathways via receptor proteins, such as TGF-β and Wnt [26, 27, 29, 31]. In colonic MPC, PDCs, and TBs, the aberrant expression of E-cadherin and β-catenin has been indicated [3, 6, 7, 12–14]. The E-cadherin and β-catenin immunoreactivities of the tumor tissue sections were compared with those of the cryptal epithelium in the noncancerous part, respectively [33].

**Routine Pathological Findings**

Macroscopic examination revealed an ulcerated tumor measuring 2.2 × 2.6 cm (Fig. 1a). Microscopically, the tumor invaded the subserosa, but no serosal exposure was noted. The tumor consisted mainly of PDCs with empty spaces (Fig. 1b); furthermore, although the glandular growth pattern of the AC was mixed, it decreased toward the invasion front (Fig. 1c). In many of the tumor glands (TGs), tumor cell detachment from the stroma toward the gland interior was observed as well as various
sized empty spaces (Fig. 1d). Furthermore, the destruction of the glandular structures was scattered, and PDCs were found near the ruptured ones (Fig. 1d). In the deep layer of the tumor, especially at the invasion front, small PDCs composed of ten or less cancer cells and TBs were scattered (Fig. 2a), regardless of the tumor center and the tumor margin. PDCs and TBs were also found in the upper and middle layers of the tumor (Fig. 1c). However, when they were counted in 30 microscopic fields (10 fields each per section), each in the upper and middle layers and in the deep layer, including the invasion front, they were found to be predominant in the deep layer [interquartile ranges for upper and middle layers vs. deep layer: PDCs, 6.0 (4.0–9.0) vs. 12.0 (10.0–13.0), \( P < 0.0001 \); TBs, 3.0 (2.0–4.0) vs. 6.0 (5.0–8.8), \( P < 0.0001 \)]. The analyses were performed by Mann–Whitney U-test using EasyR (EZR) on R commander (version 1.41) (Division of Hematology, Saitama Medical Center, Jichi Medical University, Saitama, Japan). The maximum numbers of PDCs and TBs were 17 and 13 per field, respectively, and each number corresponded to a score of 3 [9, 10], with the PDCs being predominant. The tumor metastasized to six regional lymph nodes, corresponding to pathological stage IIIB (pT3N2aM0) [34].

Notably, apoptotic cells and bodies were scattered in the TGs as were non-small-sized PDCs in the middle and deep layers of the tumor (Fig. 2b). Additionally, many of their empty spaces also contained apoptotic cells and bodies, and some of the spaces showed septum-like structures, representing a concentrated vacuole-like appearance (Fig. 2b).
Immunohistochemical Findings

D2-40

Most of the empty spaces were negative for D2-40, indicating the absence of lymphatic vessels.

M30

The M30-positive parts were distributed predominantly in the deep layer of the tumor, in a band-like appearance (Fig. 3a), whereas their distribution in the upper layer was minimal (Fig. 3a). Furthermore, they were located mainly at both the peripheries of the TGs and large PDCs and within their empty spaces and consisted of cells and granules (probably apoptotic cell debris) of various sizes (Fig. 3b). Some of the M30-positive parts within the empty spaces partially rimmed the individual vacuoles (Fig. 3c). In the small PDCs and TBs, the M30-positive parts were diminished and barely evident, especially in the TBs (Fig. 3d). The same was true for the TBs in the upper and middle layers of the tumor.

EMA

The PDCs showed linear positivity for EMA at the stroma-facing surfaces, but most of the positivity was not circumferential (Fig. 4a). Even in the TGs that partially lacked contact with the stroma, linear positivity was found at the stroma-facing surfaces of the detached tumor cells as well as at their luminal surfaces (Fig. 4b). By contrast, linear positivity for EMA was weak in the small PDCs and TBs.

TGF-β1

In both the TGs with empty spaces and the PDCs, granular cytoplasmic positivity for TGF-β1 was found (Fig. 4c). The positivity in those tumor components was observed, regardless of their locations, both states (cells or granules), and amount of M30-positive parts. By contrast, the TBs showed only faint positivity. Inflammatory cells, including neutrophils and macrophages, also showed cytoplasmic positivity for TGF-β1.

E-cadherin

The PDCs and TBs displayed marked reductions in their membranous positivity for E-cadherin, showing a dot-like appearance. Some of the TBs showed loss of the positivity. In TGs with empty spaces, the membranous positivity was moderately diminished.

β-catenin

The PDCs and TBs displayed marked reductions in their membranous positivity for β-catenin, showing a dot-like appearance (Fig. 4d). Some of the TBs showed a loss of the positivity. However, no nuclear positivity was evident in any

Fig. 3 Immunohistochemical findings. Analysis of staining the apoptotic marker M30 Cyto-DEATH. a Numerous positive areas distributed in the deep layer of the tumor (arrows). Asterisks indicate colonic muscularis propria. Scale bar: 400 μm. b Weakly positive areas inside the tumor cell nests compared to the periphery. Scale bar: 100 μm. c Positive areas, partially surrounding individual vacuoles (arrows). Scale bar: 50 μm. d Many tumor buds without a positive reaction (arrows). Scale bar: 100 μm.
of the tumor cells (Fig. 4d). In the TGs with empty spaces, the membranous positivity was moderately diminished.

**Discussion**

The distribution of M30-positive cells was not uniform, and there were differences depending on the morphology of the tumor components. M30 positivity was the least in the TBs among the tumor components. The TBs were considered to be the tumor component that had acquired the most apoptosis resistance, which was in line with previous reports [17, 18]. PDCs were also observed in the upper and middle layers of the tumor, i.e., in an old lesion in which tumorigenesis had occurred. However, there were fewer M30-positive cells in the old lesion than in the deep layer of the tumor, i.e., in a lesion newer than the upper and middle layers of the tumor. It is possible that at least some of the PDCs in the old lesion had acquired apoptosis resistance [17, 20]. These findings above suggest that the results of M30 immunostaining reflect the different stages in the apoptosis resistance-acquiring process of the tumor cells. PDCs in the deep layer of the tumor in the present case had poor ability to promptly acquire apoptosis resistance [17, 20].

In the present case, apoptosis was also scattered at the periphery of the TGs. We had previously reported a case of invasive mammary MPC with frequent apoptosis, which suggested the involvement of the apoptotic process in the formation of a micropapillary pattern in cancers [24]. The TGs of the mammary MPC had the following pathological findings in common with those of the colonic MPC in the present case: scattered tumor cell detachment from the stroma toward the gland interior; scattered empty spaces, including apoptotic cells and bodies; and M30-positive vacuoles within the empty spaces, suggesting its derivation from a secondary necrosis followed by apoptosis [24, 35]. In our previous report of invasive mammary MPC [24], we considered that apoptosis is involved in the formation of a micropapillary pattern by the following mechanisms: (1) both frequent apoptosis and secondary necrosis disturb the apical–basal polarity of the tumor cells everywhere, resulting in a condition resembling to partially polarized structures with dysplastic features [36]; (2) in the state where the basal polarity was lost due to cell detachment, even detached tumor cells without luminal differentiation might acquire an apical polarity on the stroma-facing surface, under the effects of cell–cell contacts with the neighboring tumor cells with an apical polarity [36]; and (3) secondary necrosis of the apoptotic tumor cells with luminal differentiation has the potential to partially create detached tumor cells with reverse polarity [35, 36]. In general, cells detached from the surrounding stroma fall into apoptosis [15]. In the present case, it is likely that tumor cell detachment had occurred sporadically in many TGs as a result of some unknown factor(s), resulting in frequent apoptosis [15]. Additionally, it is possible that progressive cell detachment and the subsequent disruption of the glandular structure, attributed to frequent apoptosis, caused a loss of apical–basal polarity, and the
connection between the luminal surface and the stroma-facing surface, in the tumor cells of the TG, resulting in the formation of PDCs within the empty spaces [24]. In the deep layer of the tumor in the present case, further tumor cells may have shaved off from the PDCs that had a poor ability to promptly acquire apoptosis resistance, resulting in the production of smaller PDCs and more TBs [17, 20, 24]. Taken together, the results suggest that although apoptosis is generally regarded as a cellular phenomenon that functions contrary to MPC/PDCs and TBs [17–20], it could in fact potentially reciprocally produce the tumor features in the present case [24].

In the present case, TGF-β1 also has the potential to be related to the occurrence of MPC/PDCs. By contrast, nuclear β-catenin signaling is unlikely to be activated, despite the marked reduction in the membranous expression of both E-cadherin and β-catenin. However, this deficiency in nuclear β-catenin expression is consistent with that in a previous report [3]. The TGF-β pathway plays an important role in orchestrating the EMT mechanisms [26, 27, 31] and is also involved in the downregulation of membranous E-cadherin [26, 27]. TGF-β also activates several downstream pathways mediated by effectors and/or transcription factors other than β-catenin [26, 27, 31]. Additionally, although TGF-β1 is known to induce apoptosis resistance in cells, thereby triggering the loss of cell polarity attributed to apoptosis, it has also been shown to promote the apoptotic process, indicating its dual functions with regard to this cell death phenomenon [37–39].

We have previously reported two cases of conventional CRC with high frequency of TBs and PDCs [40]. Retrospective examinations of these two CRCs revealed that apoptosis was rare and that M30 positivity in the TGs, albeit scattered, was barely evident in the PDCs and TBs. Additionally, in these two CRCs, aberrant nuclear β-catenin positivity was found in the PDCs and TBs as well as in the TGs giving rise to TBs and PDCs, regardless of TGF-β1 immunoreactivity, suggesting the involvement of nuclear β-catenin signaling in tumor budding [12–14]. These findings are in line with previous reports on PDCs and TBs in a conventional CRC [12–14, 17, 18, 20]. In a conventional CRC, unlike colonic MPC, tumor cells are considered to have already acquired resistance to apoptosis at an early stage of TB and PDC development [17, 18, 20].

This case report has some limitations, including the limited conclusive relationships that can be drawn through TGF-β1, E-cadherin, and β-catenin immunostaining alone and the intrinsic limitations of a case report. Further investigations are needed to confirm our suppositions.

In conclusion, we have described a case of MPC with frequent apoptosis that occurred mainly in the deep layer of the tumor where significantly higher numbers of TBs and PDCs were evident. The tumor components in the area appeared to have a poor ability to acquire apoptosis resistance promptly [17, 20]. This case highlights that much is still not known about the role that apoptosis plays in the pathology of cancer. Whereas most studies have pointed to the fact that MPC and TBs are simply apoptosis-resistant tumor features, this case suggests that apoptosis has the potential to reciprocally produce MPC/PDCs and TBs through the involvement of TGF-β1 [37–39, 41].

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Availability of Data and Material The authors declare that all relevant data are included in this published article and are available within the paper.

Declarations

Ethics Approval and Consent to Participate This case report was approved by the Ethics committee of Shizuoka General Hospital (approval number: SGH IRB#20180911/1). Written informed consent was obtained from the patient for publication of this case report and accompanying images.

Consent for Publication Written informed consent to publish the case details was obtained from the patient.

Conflict of Interest The authors declare no competing interests.

References

1. WHO classification of tumours editorial board, editor. WHO classification of tumours. 5th ed. France: Lyon; 2018. p. 181.
2. Verdu M, Roman R, Calvo M, Rodón N, García B, González M, Vidal A, Puig X. Clinicopathological and molecular characterization of colorectal micropapillary carcinoma. Mod Pathol. 2011;24:729–38. https://doi.org/10.1038/modpathol.2011.1.
3. Guzinsk a-Ustymowicz K, Niewiarowska K, Pryczynicz A. Invasive micropapillary carcinoma: a distinct type of adenocarcinoma in the gastrointestinal tract. World J Gastroenterol. 2014;20:4597–606. https://doi.org/10.3748/wjg.v20.i16.4597.
4. Gonzalez RS, Huh WJ, Cates JMM, Washington K, Beauchamp RD, Coffey RJ, Shi C. Micropapillary colorectal carcinoma: clinical, pathological and molecular properties, including evidence of epithelial-mesenchymal transition. Histopathology. 2017;70:223–231. https://doi.org/10.1111/his.13068.
5. Nassar H, Pansare V, Zhang H, Che M, Sakr W, Ali-Fehmi R, Grignon D, Sarkar F, Cheng J, Adsay V. Pathogenesis of invasive micropapillary carcinoma: role of MUC1 glycoprotein.
6. Barresi V, Branca G, Vitarelli E, Tuccari G. Micropapillary pattern and poorly differentiated clusters represent the same biological phenomenon in colorectal cancer: a proposal for a change in terminology. Am J Clin Pathol. 2014;142:375–83. https://doi.org/10.1093/ajcp/pfu027.

7. Hong M, Kim JW, Shin MK, Kim BC. Poorly differentiated clusters in colorectal adenocarcinomas share biological similarities with micropapillary patterns as well as tumour buds. J Korean Med Sci. 2017;32:1595–602. https://doi.org/10.3344/jkms.2017.32.10.1595.

8. Shivji S, Conner JR, Barresi V, Kirsch R. Poorly differentiated clusters in colorectal cancer: a current review and implications for future practice. Histopathology. 2020;77:351–68. https://doi.org/10.1111/his.14128.

9. Ueno H, Kajiwara Y, Shimazaki H, Shinto E, Hashiguchi Y, Nakanishi K, Maekawa K, Katsurada Y, Nakamura T, Mochizuki U, Yamamoto J, Hase K. New criteria for histologic grading of colorectal cancer. Am J Surg Pathol. 2012;36:193–201. https://doi.org/10.1097/PAS.0b013e3182335edeec.

10. Lugli A, Kirsch R, Ajioya Y, Bosman F, Cathomas G, Dawson H, El Zimaity H, Flejou JF, Hansen TP, Hartmann A, Kakar S, Langner C, Nagtegaal I, Puppa G, Riddell R, Ristimäki A, Sheahan K, Smyrk T, Sugihara K, Terris B, Ueno H, Vieth M, Zlobec I, Quirke P. Recommendations for reporting tumor budding in colorectal cancer based on the international tumor budding consensus conference (ITBCC) 2016. Mod Pathol. 2017;30:1299–31. https://doi.org/10.1038/modpathol.2017.46.

11. Yang M, Rehman AU, Zuo C, Sheehan CE, Lee EC, Lin J, Zhao Z, Choi E, Lee H. A novel histologic grading scheme based on poorly differentiated clusters is applicable to treated rec-

12. Grigore AD, Jolly MK, Jia D, Farach-Carson MC, Levine H. Tumor budding: the name is EMT. Partial EMT J Clin Med. 2016;5:51. https://doi.org/10.3390/jcm5050051.

13. Bertoni L, Barresi V, Bonetti LR, Caramaschi S, Mangogna A, Lionti S, Azzoni P, Carnevale G, Pisciotta A, Salvatici T. Poorly differentiated clusters (PDC) in colorectal cancer: does their localization in tumor matter? Ann Diagn Pathol. 2019;41:106–11. https://doi.org/10.1016/j.aadpath.2019.06.008.

14. Lugli A, Zlobec I, Berger MD, Kirsch R, Nagtegaal ID. Tumor budding in solid cancers. Nat Rev Clin Oncol. 2021;18:101–5. https://doi.org/10.1038/s41571-020-0422-y.

15. Frisch SM, Schaller M, Cieply B. Mechanisms that link the oncogenic epithelial-mesenchymal transition to suppression of anoikis. J Cell Sci. 2013;126:21–9. https://doi.org/10.1242/jcs.120907.

16. Paoli P, Giannoni E, Chiarugi P. Anoikis molecular pathways and its role in cancer progression. Biochim Biophys Acta. 2013;1833:3481–98. https://doi.org/10.1016/j.bbamcr.2013.06.026.

17. Dawson H, Koelzer VH, Karamitiopoulou E, Economou M, Hammer C, Muller DE, Lugli A, Zlobec I. The apoptotic and proliferation rate of tumour budding cells in colorectal cancer outlines a heterogeneous population of cells with various impacts on clinical outcome. Histopathology. 2014;64:577–84. https://doi.org/10.1111/his.12294.

18. Dawson H, Grundmann S, Koelzer VH, Galván JA, Kirsch R, Karamitiopoulou E, Lugli A, Inderbitzin D, Zlobec I. Tyrosine kinase receptor B (TrkB) expression in colorectal cancers highlights anoikis resistance as a survival mechanism of tumour budding cells. Histopathology. 2015;66:715–25. https://doi.org/10.1111/his.12603.
34. Brierley JD, Gospodarowicz MK, Wittekind C. Union for International Cancer Control (UICC): TNM classification of malignant tumours. 8th ed. New Jersey: John Wiley & Sons; 2017.

35. Zhang Y, Chen X, Gueydan C, Han J. Plasma membrane changes during programmed cell deaths. Cell Res. 2018;28:9–21. https://doi.org/10.1038/cr.2017.133.

36. Manninen A. Epithelial polarity-generating and integrating signals from the ECM with integrins. Exp Cell Res. 2015;334:337–49. https://doi.org/10.1016/j.yexcr.2015.01.003.

37. Leight JL, Wozniak MA, Chen S, Lynch ML, Chen CS. Matrix rigidity regulates a switch between TGF-β1-induced apoptosis and epithelial-mesenchymal transition. Mol Biol Cell. 2012;23:781–91. https://doi.org/10.1091/mbc.E11-06-0537.

38. David CJ, Huang YH, Chen M, Su J, Zou Y, Bardeesy N, Iacobuzio-Donahue CA, Massagué J. TGF-β tumor suppression through a lethal EMT. Cell. 2016;164:1015–30. https://doi.org/10.1016/j.cell.2016.01.009.

39. Zhang Y, Alexander PB, Wang XF. TGF-β family signaling in the control of cell proliferation and survival. Cold Spring Harb Perspect Biol. 2017;9:a022145. https://doi.org/10.1101/cshperspect.a022145.

40. Arai K, Ishimatsu H, Iwasaki T, Tsuchiya C, Sonoda A, Ohata K. Membranous S100A10 involvement in the tumor budding of colorectal cancer during oncogenesis: report of two cases with immunohistochemical analysis. World J Surg Oncol. 2020;18:289. https://doi.org/10.1186/s12957-020-02075-4.

41. Derynck R, Akhurst RJ, Balmain A. TGF-beta signaling in tumor suppression and cancer progression. Nat Genet. 2001;29:117–29. https://doi.org/10.1038/ng1001-117.

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