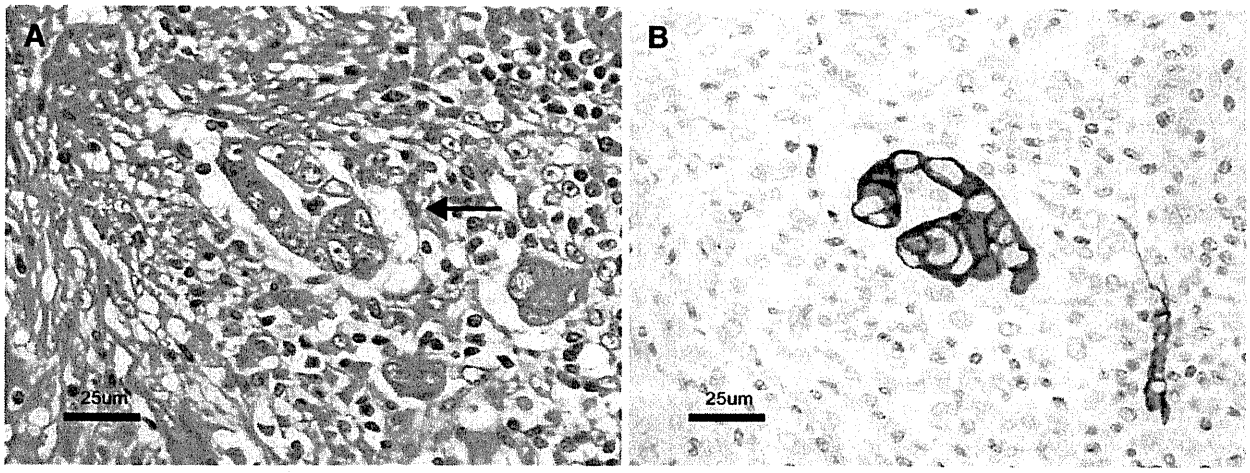

Fig. 14 Space formed by artifacts during preparation of the specimen (*arrow* in **a**). **a** A cancer cell nest is visible in the interstitial space. **b** Double staining for cytokeratin and D2-40. The interstitial space is D2-40-negative

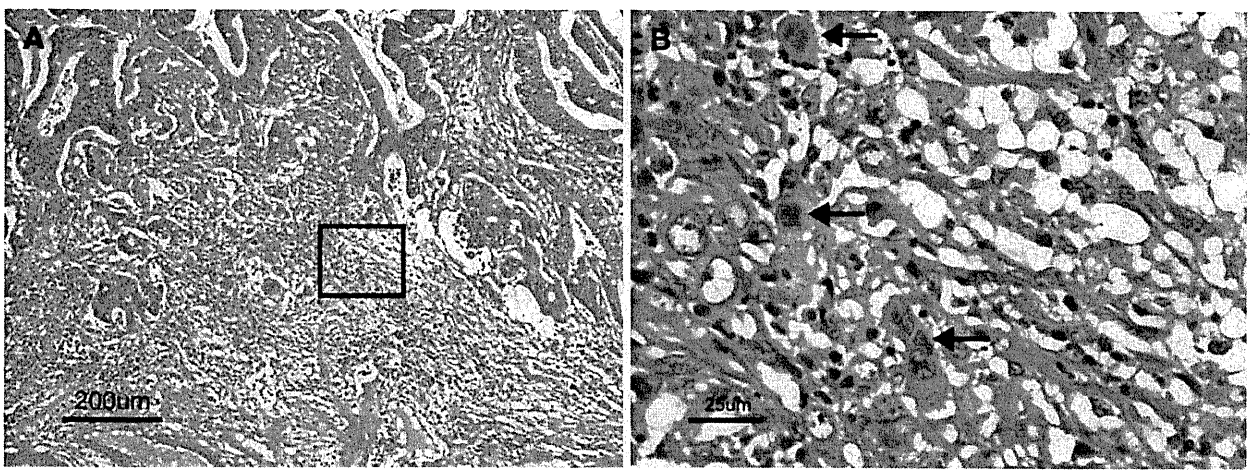

Fig. 15 Budding (*arrows* in **b**). **a** A cancer cell nest consisting of 1 or less than 5 cells that has infiltrated the interstitium at the invasive margin of the cancer is seen. **b** is the square area in **a**

Table 11 Depth of invasion of sm cancer and lymph node metastasis (modified from [80])

sm invasion distance (μm)	Pedunculated		Nonpedunculated	
	Number of lesions	*n* (+) (%)	Number of lesions	*n* (+) (%)
Head invasion	53	3 (5.7)		
$0 < X < 500$	10	0 (0)	65	0 (0)
$500 \leq X < 1{,}000$	7	0 (0)	58	0 (0)
$1{,}000 \leq X < 1{,}500$	11	1 (9.1)	52	6 (11.5)
$1{,}500 \leq X < 2{,}000$	7	1 (14.3)	82	10 (12.2)
$2{,}000 \leq X < 2{,}500$	10	1 (10.0)	84	13 (15.5)
$2{,}500 \leq X < 3{,}000$	4	0 (0)	71	8 (11.3)
$3{,}000 \leq X < 3{,}500$	9	2 (22.2)	72	5 (6.9)
$3{,}500 \leq X$	30	2 (6.7)	240	35 (14.6)

The lymph node metastasis rate of patients with a depth of invasion of 1,000 μm or above was 12.5%

All 3 lymph node metastasis-positive patients with head invasion were ly positive

Side Memo 1

- Method for measuring depth of SM invasion (Fig. 11):

– When it is possible to identify or estimate the location of the muscularis mucosae, depth of SM invasion is measured from the lower border of the muscularis mucosae of the lesion, regardless of the macroscopic type.
– When it is not possible to identify or estimate the location of the muscularis mucosae, the depth of SM invasion is measured from the surface of the lesion. The phrase "possible to identify or to estimate" means that there is no "deformity" (i.e., disarray, dissection, rupture, fragmentation, etc.) of the muscularis mucosae as a result of SM invasion. If a deformed muscularis mucosa is used as the baseline of the measurement, the depth of SM invasion may be underestimated. Although judging whether there is a "deformity" is not always straightforward, if a desmoplastic reaction is present around the muscularis mucosae, it is assumed to be "deformed."
– For pedunculated lesions with a tangled muscularis mucosae, depth of SM invasion is measured as the distance between the point of deepest invasion and the reference line, which is defined as the boundary between the tumor head and the stalk (the boundary between the tumor area and the non-tumor area in the mucosa). Invasion by pedunculated lesions that is limited to within the head is defined as "head invasion."

- Method for assessing vascular invasion (Figs. 12, 13, 14):

– Attention to arteries is a key factor in assessing venous invasion. Venous invasion is highly likely when a circular, semicircular, or oblong cancer cell nest with regular margins is located in the vicinity of an artery and distant from the main lesion. If such a cancer cell nest is surrounded by venous wall structures (such as internal elastic membrane or perivascular smooth muscle), it can be concluded to represent venous invasion. However, the venous wall structures are often displaced or obliterated by the cancer cell nest, and it is difficult to recognize in hematoxylin and eosin stained sections.
– The presence of cancer cells and cancer cell nests in the interstitial space suggests lymphatic invasion. A space filled with lymph and lymphocytes is especially likely to be a lymph vessel. When endothelial cells are identified around the space, the space can be concluded to represent a lymph vessel. However, it is often difficult to identify endothelial cells in specimens stained with hematoxylin and eosin, and spaces may be artifacts created during the process of preparing the specimen.
– As stated above, evaluation of vascular invasion, which is an important indicator for determining treatment strategies for SM cancer, is often difficult in hematoxylin and eosin stained specimens. Special staining methods are useful for evaluating vascular invasion, such as elastica van Gieson staining or Victoria blue staining for venous invasion, and D2-40 immunostaining for lymphatic invasion.

- Method for assessing tumor budding (Fig. 15):

[Definition of tumor budding] [79]

A cancer cell nest consisting of 1 or less than 5 cells that infiltrates the interstitium at the invasive margin of the cancer.

[Grade of budding]

After selecting one field where budding is the most intensive, the number of buddings is counted in a field measuring 0.785 mm^2 observed through a 20× objective lens (WHK 10× ocular lens). Depending on the number of buddings, the grade of budding is defined as follows:

Grade 1: 0–4
Grade 2: 5–9
Grade 3: 10 or more

- The lymph node metastasis rate associated with grade 2/3 tumors is significantly higher than that associated with grade 1 tumors. A multi-center study conducted by the Budding Investigation Project Committee (2005–) of the JSCCR in which grade 1 was defined as "low grade" and grade 2/3 as "high grade" showed that high grade is an independent predictor of lymph node metastasis.

CQ-3: Laparoscopic surgery for colorectal cancer

Recommendation: Category B

- Since laparoscopic surgery requires surgical skills that are different from those required for open abdominal surgery, and an understanding of regional anatomy is essential for laparoscopic surgery, the indication criteria should be determined depending on the skills of the surgical team.

Laparoscopic surgery is suitable for D2, D1 or D0 resection of colon and RS cancer, and is well indicated for the treatment of cStage 0 to cStage I disease. Because laparoscopic colectomy with D3 dissection is difficult, whether it is indicated for patients with cStage II to cStage III

disease should be determined after carefully considering the skills of the surgical team. Laparoscopic surgery is also difficult in patients with transverse colon cancer, in severely obese patients, and in patients with severe adhesions. The efficacy and safety of laparoscopic surgery for rectal cancer has not been sufficiently established.

CQ-4: Resection of the primary tumor in patients with unresectable distant metastases

Recommendation: Category B

- The initial resection of the primary tumor should be determined based on the performance status of each patient, such as the symptoms caused by the primary tumor, the status of distant metastases, and the patient's general condition. Resection of the primary tumor is often desirable when a patient has symptoms caused by the primary tumor that cannot be well controlled by other therapies, if the patient is sufficiently able to tolerate surgery, and the resection can be accomplished with acceptable morbidity.

CQ-5: Resection of peritoneal metastases (carcinomatous peritonitis)

Recommendation: Category C

- If patients with localized peritoneal dissemination (P1, P2) have no other unresectable distant metastases and resection will not result in excessive invasion, it is preferable to resect the disseminated tumors at the same time as the resection of the primary tumor.

CQ-6: Surgical treatment for local recurrence of rectal cancer

Recommendation: Category B

- Resection should be considered for local recurrence of rectal cancer when R0 resection is considered possible.

CQ-7: Resection in patients with liver and lung metastases

Recommendation: Category C

- The efficacy of resection in patients who have liver and lung metastases at the same time has been shown, and thus resection should be considered for patients with resectable liver and lung metastases.

However, there are insufficient data to determine the indication criteria for surgery. It is necessary to obtain informed consent after informing the patient of the rather low cure rate and the absence of outcome predictors.

CQ-8: Adjuvant chemotherapy after curative resection of liver metastases

Recommendation: Category B

- The efficacy of adjuvant chemotherapy after hepatectomy has not been established. It is desirable to investigate its efficacy in clinical trials.

CQ-9: Preoperative chemotherapy for resectable liver metastases

Recommendation: Category B

- The safety of preoperative chemotherapy for resectable liver metastases has not been established. It should be evaluated in properly designed clinical trials.

CQ-10: Chemotherapy for unresectable liver metastases

Recommendation: Category B

- Hepatectomy should be considered for liver metastases that have become resectable after successful chemotherapy.

No clear difference has been observed between hepatic arterial infusion therapy and systemic chemotherapy in terms of the prolongation of survival time of patients with unresectable liver metastases.

CQ-11: Postoperative adjuvant chemotherapy and age

Recommendation: Category A

- Even in patients 70 years old or older, postoperative adjuvant chemotherapy can be performed if their PS is good, if the function of major organs is adequate, and if there are no complications that may be a risk for performing chemotherapy.

CQ-12: Postoperative adjuvant chemotherapy for stage II colorectal cancer

Recommendation: Category A

- The usefulness of postoperative adjuvant chemotherapy for stage II colorectal cancer has not been proven, and it is not appropriate to routinely administer adjuvant chemotherapy to all patients with stage II colorectal cancer.

CQ-13: Duration of postoperative adjuvant chemotherapy

Recommendation: Category A

- Although no definitive conclusion regarding the duration of postoperative adjuvant chemotherapy has been reached, the current standard duration of treatment by 5-FU-based adjuvant chemotherapy is 6 months.

CQ-14: Oxaliplatin (L-OHP) in postoperative adjuvant chemotherapy

Recommendation: Category A

- In August 2009, L-OHP was approved for postoperative adjuvant chemotherapy in Japan. When selecting target patients, the indication should be determined after obtaining sufficient informed consent regarding adverse events and medical care costs as well as the expected additional benefit in terms of survival time.

CQ-15: Molecular target drugs for secondary treatment

Recommendation: Category B

- It is desirable to use bevacizumab as secondary treatment in patients who can be treated with bevacizumab and have not received it as primary treatment. There is no clear evidence supporting the optimal dose in this situation (5 or 10 mg/kg) [44, 49].

CQ-16: KRAS gene mutations and anti-EGFR antibody drugs

Recommendation: Category A

- The usefulness of anti-EGFR antibody drugs has been reported in metastatic colorectal cancer without KRAS gene mutations [38–41, 47, 53, 55, 85–90].

Side Memo 2

- Anti-EGFR antibody drugs and EGFR immunostaining

Since most clinical research on cetuximab has been conducted on EGFR-positive patients, insurance coverage is limited to EGFR-positive patients. On the other hand, most clinical research on panitumumab has also been conducted on EGFR-positive patients, and evidence in regard to EGFR-negative patients is insufficient, but insurance coverage has been restricted to EGFR-positive patients. A recent report showed that there is no relationship between the effect of anti-EGFR antibody drugs and the level of EGFR expression assessed by immunostaining [91].

- CPT-11 and UGT1A1 genetic polymorphism

SN-38 is an active metabolite of CPT-11 and the UGT1A1 gene encodes an intrahepatic metabolizing enzyme which converts the active form SN-38 to the inactive form SN-38 G. In patients who are double heterozygotes for *6 and *28 or homozygotes for *6 or *28 of the UGT1A1 gene, the glucuronic acid conjugation capacity of UGT1A1 is known to be decreased and the metabolism of SN-38 to be delayed, and serious adverse drug reactions such as neutropenia may occur as a result. It is especially desirable to test for a UGT1A1 genetic polymorphism before administering CPT-11 to patients with a high serum bilirubin level, elderly patients, patients whose general condition is poor (e.g., PS2), and patients in whom severe toxicity (especially neutropenia) developed after the last administration of CPT-11. On the other hand, because CPT-11 toxicity cannot be predicted with certainty on the basis of the presence of a UGT1A1 genetic polymorphism alone, it is essential to monitor the patient's general condition during treatment and manage adverse drug reactions carefully regardless of whether a genetic polymorphism is detected.

CQ-17: Significance of preoperative chemoradiotherapy for rectal cancer

Recommendation: Category C

- Preoperative chemoradiotherapy is standard treatment for rectal cancer in Europe and the United States. However, there is insufficient evidence in support of its efficacy and safety in Japan, and it needs to be evaluated in properly designed clinical trials.

CQ-18: Chemoradiotherapy for unresectable locally advanced and locally recurrent rectal cancer

Recommendation: Category C

- The indication for chemoradiotherapy aiming at complete cure by R0 resection will also be considered for locally advanced or locally recurrent, unresectable rectal cancer.

CQ-19: Significance of surveillance after surgery of colorectal cancer

19A: Diagnosis of recurrence
Recommendation: Category A

- Early detection of recurrence has been shown to contribute to an improvement in outcome, and

postoperative surveillance examinations should be performed regularly. However, an optimal surveillance protocol incorporating the health economical point of view has not been sufficiently established.

19B: Multiple cancer
Recommendation: Category B

- With the exception of hereditary colorectal cancer, a past medical history of colorectal cancer has not been demonstrated to be a risk factor for the development of cancer in other organs, and it is unnecessary to incorporate special surveillance for multiple cancer into the surveillance performed after curative surgery for colorectal cancer.

References

1. Japanese Society for Cancer of the Colon and Rectum (2010) JSCCR guidelines 2010 for the treatment of colorectal cancer. Kanehara & Co., Ltd., Tokyo
2. Kudo S (1993) Endoscopic mucosal resection of flat and depressed early colorectal cancer. Endoscopy 25:455–461
3. Tanaka S, Oka S, Chayama K (2008) Colorectal endoscopic submucosal dissection (ESD): the present status and future perspective including its differentiation from endoscopic mucosal resection (EMR). J Gastroenterol 43:641–651
4. Japanese Society for Cancer of the Colon and Rectum (2010) Multi-Institutional Registry of Large Bowel Cancer in Japan. Cases treated in 1995–1998 [vols. 17 (1999), 18 (2000), 21 (2001), 24 (2003)]. Japanese Society for Cancer of the Colon and Rectum, Tokyo
5. Heald RJ, Husband EM, Ryall RD (1982) The mesorectum in rectal cancer surgery—the clue to pelvic recurrence? Br J Surg 69:613–616
6. MacFarlane JK, Ryall RD, Heald RJ (1993) Mesorectal excision for rectal cancer. Lancet 341:457–460
7. Enker WE, Thaler HT, Cranor ML et al (1995) Total mesorectal excision in the operative treatment of carcinoma of the rectum. J Am Coll Surg 181:335–346
8. Lowry AC, Simmang CL, Boulos P et al (2001) Consensus statement of definitions for anorectal physiology and rectal cancer. Dis Colon Rectum 44:915–919
9. Sugihara K, Kobayashi H, Kato T et al (2006) Indication and benefit of pelvic sidewall dissection for rectal cancer. Dis Colon Rectum 49:1663–1672
10. Japanese Society for Cancer of the Colon and Rectum (1998) General rules for clinical and pathological studies on cancer of the colon, rectum and anus, 6th edn. Kanehara & Co., Ltd., Tokyo
11. Mentges B, Buess G, Schafer D et al (1996) Local therapy of rectal tumors. Dis Colon Rectum 39:886–892
12. Murata S, Moriya Y, Akasu T et al (1998) Resection of both hepatic and pulmonary metastases in patients with colorectal carcinoma. Cancer 83:1086–1093
13. Robinson BJ, Rice TW, Strong SA et al (1999) Is resection of pulmonary and hepatic metastases warranted in patients with colorectal cancer? J Thorac Cardiovasc Surg 117:66–76
14. Lambert LA, Colacchio TA, Barth RJ Jr (2000) Interval hepatic resection of colorectal metastases improves patient selection. Arch Surg 135:473–479
15. Kobayashi K, Kawamura M, Ishihara T (1999) Surgical treatment for both pulmonary and hepatic metastases from colorectal cancer. J Thorac Cardiovasc Surg 118:1090–1096
16. Regnard JF, Grunenwald D, Spaggiari L et al (1998) Surgical treatment for hepatic and pulmonary metastases from colorectal cancers. Ann Thorac Surg 66:214–218
17. Patchell RA, Tibbs PA, Walsh JW et al (1990) A randomized trial of surgery in the treatment of single metastases to the brain. N Engl J Med 322:494–500
18. National Institute of Health Consensus Conference (1990) Adjuvant therapy for patients with colon and rectal cancer. JAMA 264:1444–1450
19. Benson AB 3rd, Schrag D, Somerfield MR et al (2004) American Society of Clinical Oncology recommendations on adjuvant chemotherapy for stage II colon cancer. J Clin Oncol 22:3408
20. Van Cutsem E, Oliveira J, ESMO Guidelines Working Group (2008) Colon cancer: ESMO clinical recommendations for diagnosis, adjuvant treatment and follow-up. Ann Oncol 19(Suppl 2):ii29–ii30
21. André T, Boni C, Mounedji-Boudiaf L et al, Multicenter International Study of Oxaliplatin/5-Fluorouracil/Leucovorin in the Adjuvant Treatment of Colon Cancer (MOSAIC) Investigators (2004) Oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment for colon cancer. N Engl J Med 350:2343–2351
22. Kuebler JP, Wieand HS, O'Connell MJ et al (2007) Oxaliplatin combined with weekly bolus fluorouracil and leucovorin as surgical adjuvant chemotherapy for stage II and III colon cancer: results from NSABP C-07. J Clin Oncol 25:2198–2204
23. André T, Boni C, Navarro M et al (2009) Improved overall survival with oxaliplatin, fluorouracil, and leucovorin as adjuvant treatment in stage II or III colon cancer in the MOSAIC trial. J Clin Oncol 27:3109–3116
24. Wolmark N, Wieand S, Kuebler PJ et al (2008) A phase III trial comparing FULV to FULV+oxaliplatin in stage II or III carcinoma of the colon: survival results of NSABP Protocol C-07. Proc Am Soc Clin Oncol 26 (Abstr LBA4005)
25. Haller D, Tabernero J, Maroun J et al (2009) First efficacy findings from a randomized phase III trial of capecitabine + oxaliplatin vs. bolus 5-FU/LV for stage III colon cancer (NO16968/XELOXA study). In: Proc ECCO 15–34th ESMO Congr, Berlin, Germany, 20–24 Sept 2009, Abstr 5LBA
26. Haller DG, Catalano PJ, Macdonald JS et al (2005) Phase III study of fluorouracil, leucovorin, and levamisole in high-risk stage II and III colon cancer: final report of Intergroup 0089. J Clin Oncol 23:8671–8678
27. Scheithauer W, Rosen H, Kornek GV et al (1993) Randomised comparison of combination chemotherapy plus supportive care with supportive care alone in patients with metastatic colorectal cancer. Br Med J 306:752–755
28. Simmonds PC (2000) Palliative chemotherapy for advanced colorectal cancer: systematic review and meta-analysis. Colorectal Cancer Collaborative Group. Br Med J 321:531–535
29. Cunningham D, Pyrhonen S, James RD et al (1998) Randomised trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. Lancet 352:1413–1418
30. de Gramont A, Figer A, Seymour M et al (2000) Leucovorin and fluorouracil with or without oxaliplatin as first-line treatment in advanced colorectal cancer. J Clin Oncol 18:2938–2947
31. Goldberg R, Sargent D, Morton R et al (2004) A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 22:23–30
32. Saltz LB, Clarke S, Díaz-Rubio E et al (2008) Bevacizumab in combination with oxaliplatin-based chemotherapy as first-line therapy in metastatic colorectal cancer: a randomized phase III study. J Clin Oncol 26:2013–2019

33. Cassidy J, Clarke S, Díaz-Rubio E et al (2008) Randomized phase III study of capecitabine plus oxaliplatin compared with fluorouracil/folinic acid plus oxaliplatin as first-line therapy for metastatic colorectal cancer. J Clin Oncol 26:2006–2012
34. Tournigand C, André T, Achille E et al (2004) FOLFIRI followed by FOLFOX6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR Study. J Clin Oncol 22:229–237
35. Douillard JY, Cunningham D, Roth AD et al (2000) Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 355:1041–1047
36. Fuchs CS, Marshall J, Mitchell E et al (2007) Randomized, controlled trial of irinotecan plus infusional, bolus, or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: results from the BICC-C Study. J Clin Oncol 25:4779–4786
37. Fuchs CS, Marshall J, Barrueco J et al (2008) Randomized, controlled trial of irinotecan plus infusional, bolus or oral fluoropyrimidines in first-line treatment of metastatic colorectal cancer: updated results from the BICC-C study. J Clin Oncol 26:689–690
38. Bokemeyer C, Bondarenko I, Makhson A et al (2009) Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 27:663–671
39. Siena S, Cassidy J, Tabernero J et al (2010) Randomized phase III study of panitumumab (pmab) with FOLFOX4 compared to FOLFOX4 alone as first-line treatment (tx) for metastatic colorectal cancer (mCRC): PRIME trial. In: Proc 2010 ASCO Gastrointestinal Cancers Symp, Orlando, FL, USA, 22–24 Jan 2010, Abstr 283
40. Van Cutsem E, Köhne CH, Hitre E et al (2009) Cetuximab and chemotherapy as initial treatment for metastatic colorectal cancer. N Engl J Med 360:1408–1417
41. Kohne C, Mineur L, Greil R et al (2010) Primary analysis of a phase II study (20060314) combining first-line panitumumab (pmab) with FOLFIRI in the treatment of patients (pts) with metastatic colorectal cancer (mCRC). In: Proc 2010 ASCO Gastrointestinal Cancers Symp, Orlando, FL, USA, 22–24 Jan 2010, Abstr 414
42. Petrelli N, Herrera L, Rustum Y et al (1987) A prospective randomized trial of 5-fluorouracil versus 5-fluorouracil and high-dose leucovorin versus 5-fluorouracil and methotrexate in previously untreated patients with advanced colorectal carcinoma. J Clin Oncol 5:1559–1565
43. Hurwitz H, Fehrenbacher L, Hainsworth JD et al (2005) Bevacizumab in combination with fluorouracil and leucovorin: an active regimen for first-line metastatic colorectal cancer. J Clin Oncol 23:3502–3508
44. Kabbinavar F, Hurwitz HI, Fehrenbacher L et al (2003) Phase II, randomized trial comparing bevacizumab plus fluorouracil (FU)/leucovorin (LV) with FU/LV alone in patients with metastatic colorectal cancer. J Clin Oncol 21:60–65
45. Shirao K, Hoff PM, Ohtsu A et al (2004) Comparison of the efficacy, toxicity, and pharmacokinetics of a uracil/tegafur (UFT) plus oral leucovorin (LV) regimen between Japanese and American patients with advanced colorectal cancer: joint United States and Japan study of UFT/LV. J Clin Oncol 22:3466–3474
46. Sobrero AF, Maurel J, Fehrenbacher L et al (2008) EPIC: phase III trial of cetuximab plus irinotecan after fluoropyrimidine and oxaliplatin failure in patients with metastatic colorectal cancer. J Clin Oncol 26:2311–2319
47. Peeters M, Price TJ, Hotko YS et al (2010) Randomized phase III study of panitumumab (pmab) with FOLFIRI vs FOLFIRI alone as second-line treatment (tx) in patients (pts) with metastatic colorectal cancer (mCRC): patient-reported outcomes (PRO). In: Proc 2010 ASCO Gastrointestinal Cancers Symp, Orlando, FL, USA, 22–24 Jan 2010, Abstr 282
48. Rothenberg ML, Oza AM, Bigelow RH et al (2003) Superiority of oxaliplatin and fluorouracil-leucovorin compared with either therapy alone in patients with progressive colorectal cancer after irinotecan and fluorouracil-leucovorin: interim results of a phase III trial. J Clin Oncol 21:2059–2069
49. Giantonio BJ, Catalano PJ, Meropol NJ et al (2007) Bevacizumab in combination with oxaliplatin, fluorouracil, and leucovorin (FOLFOX4) for previously treated metastatic colorectal cancer: results from the Eastern Cooperative Oncology Group Study E3200. J Clin Oncol 25:1539–1544
50. Rothenberg ML, Cox JV, Butts C et al (2008) Capecitabine plus oxaliplatin (XELOX) versus 5-fluorouracil/folinic acid plus oxaliplatin (FOLFOX-4) as second-line therapy in metastatic colorectal cancer: a randomized phase III noninferiority study. Ann Oncol 19:1720–1726
51. Cunningham D, Humblet Y, Siena S et al (2004) Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 351:337–345
52. Jonker DJ, O'Callaghan CJ, Karapetis CS et al (2007) Cetuximab for the treatment of colorectal cancer. N Engl J Med 357:2040–2048
53. Karapetis CS, Khambata-Ford S, Jonker DJ et al (2008) K-ras mutations and benefit from cetuximab in advanced colorectal cancer. N Engl J Med 359:1757–1765
54. Van Cutsem E, Peeters M, Siena S et al (2007) Open-label phase III trial of panitumumab plus best supportive care compared with best supportive care alone in patients with chemotherapy-refractory metastatic colorectal cancer. J Clin Oncol 25:1658–1664
55. Amado RG, Wolf M, Peeters M et al (2008) Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 26:1626–1634
56. Skibber JM, Hoff PM, Minsky BD et al (2001) Cancer of the rectum. In: Devita VT, Hellman S, Rosenberg SA (eds) Cancer: principles and practice of oncology, 6th edn. Lippincott, Williams and Wilkins, Philadelphia, pp 1271–1318
57. Swedish Rectal Cancer Trial (1997) Improved survival with preoperative radiotherapy in resectable rectal cancer. N Engl J Med 336:980–987
58. Camma C, Giunta M, Fiorica F et al (2000) Preoperative radiotherapy for resectable rectal cancer. A meta-analysis. J Am Med Assoc 284:1008–1015
59. Colorectal Cancer Collaborative Group (2001) Adjuvant radiotherapy for rectal cancer: a systematic overview of 22 randomised trials involving 8507 patients. Lancet 358:1291–1304
60. Kapiteijn E, Marijnen CA, Nagtegaal ID et al (2001) Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med 345:638–646
61. Peeters KC, Marijnen CA, Nagtegaal ID et al (2007) The TME trial after a median follow-up of 6 years: increased local control but no survival benefit in irradiated patients with resectable rectal carcinoma. Ann Surg 246:693–701
62. Marijnen CA, van de Velde CJ, Putter H et al (2005) Impact of short-term preoperative radiotherapy on health-related quality of life and sexual functioning in primary rectal cancer: report of a multicenter randomized trial. J Clin Oncol 23:1847–1858
63. Peeters KC, van de Velde CJ, Leer JW et al (2005) Late side effects of short-course preoperative radiotherapy combined with total mesorectal excision for rectal cancer: increased bowel dysfunction in irradiated patients—a Dutch colorectal cancer group study. J Clin Oncol 23:6199–6206
64. Francois Y, Nemoz CJ, Baulieux J et al (1999) Influence of the interval between preoperative radiation therapy and surgery on

downstaging and on the rate of sphincter-sparing surgery for rectal cancer: the Lyon R90–01 randomized trial. J Clin Oncol 17:2396–2402
65. Bosset JF, Collette L, Calais G et al, EORTC Radiotherapy Group Trial 22921 (2006) Chemotherapy with preoperative radiotherapy in rectal cancer. N Engl J Med 355:1114–1123
66. Gerard JP, Conroy T, Bonnetain F et al (2006) Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3–4 rectal cancers: results of FFCD 9203. J Clin Oncol 24:4620–4625
67. Bujko K, Nowacki MP, Nasierowska-Guttmejer A et al (2006) Long-term results of a randomized trial comparing preoperative short-course radiotherapy with preoperative conventionally fractionated chemoradiation for rectal cancer. Br J Surg 93:1215–1223
68. Sauer R, Becker H, Hohenberger W, German Rectal Cancer Study Group et al (2004) Preoperative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med 351:1731–1740
69. WHO (1990) Cancer pain relief and palliative care (WHO Tech Rep Ser 804). WHO, Geneva, pp 21–22
70. Renehan AG, Egger M, Saunders MP et al (2002) Impact on survival of intensive follow up after curative resection for colorectal cancer: systematic review and meta-analysis of randomised trial. Br Med J 324:813–816
71. Figueredo A, Rumble RB, Maroun J et al (2003) Follow-up of patients with curatively resected colorectal cancer: a practice guideline. BMC Cancer 6:26
72. Renehan AG, Egger M, Saunders MP et al (2005) Mechanisms of improved survival from intensive followup in colorectal cancer: a hypothesis. Br J Cancer 92:430–433
73. Jeffery M, Hickey BE, Hider PN (2007) Follow-up strategies for patients treated for non-metastatic colorectal cancer. Cochrane Database Syst Rev 24:CD002200
74. Tjandra JJ, Chan MK (2007) Follow-up after curative resection of colorectal cancer: a meta-analysis. Dis Colon Rectum 50:1783–1799
75. Bruinvels D, Stiggelbout A, Kievit J et al (1994) Follow-up of patients with colorectal cancer. A meta-analysis. Ann Surg 219:174–182
76. Fleischer D, Goldberg S, Browning T et al (1989) Detection and surveillance of colorectal cancer. JAMA 261:580–585
77. Green RJ, Metlay JP, Propert K et al (2002) Surveillance for second primary colorectal cancer after adjuvant chemotherapy: an analysis of Intergroup 0089. Ann Int Med 136:261–269
78. Berman J, Cheung R, Weiberg D (2000) Surveillance after colorectal cancer resection. Lancet 355:395–399
79. Ueno H, Mochizuki H, Hashiguchi Y et al (2004) Risk factors for an adverse outcome in early invasive colorectal carcinoma. Gastroenterology 127:385–394
80. Kitajima K, Fujimori T, Fujii S et al (2004) Correlations between lymph node metastasis and depth of submucosal invasion in submucosal invasive colorectal carcinoma: a Japanese collaborative study. J Gastroenterol 39:534–543
81. Tanaka S, Haruma K, Oh-e H et al (2000) Conditions of curability after endoscopic resection for colorectal carcinoma with submucosally massive invasion. Oncol Rep 7:783–788
82. Japanese Society for Cancer of the Colon and Rectum (1980) General rules for clinical and pathological studies on cancer of the colon, rectum and anus, 2nd edn. Kanehara & Co., Ltd., Tokyo
83. Japanese Society for Cancer of the Colon and Rectum (1994) General rules for clinical and pathological studies on cancer of the colon, rectum and anus, 5th edn. Kanehara & Co., Ltd., Tokyo
84. Japanese Society for Cancer of the Colon and Rectum (2005) JSCCR guidelines 2005 for the treatment of colorectal cancer. Kanehara & Co., Ltd., Tokyo
85. Cappuzzo F, Finocchiaro G, Rossi E et al (2008) EGFR FISH assay predicts for response to cetuximab in chemotherapy refractory colorectal cancer patients. Ann Oncol 19:717–723
86. Lièvre A, Bachet JB, Boige V et al (2008) KRAS mutation as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 26:374–379
87. Lièvre A, Bachet JB, Corre DL et al (2006) KRAS mutation status is predictive of response to cetuximab therapy in colorectal cancer. Cancer Res 66:3992–3995
88. Khambata-Ford S, Garrett CR, Meropol NJ et al (2007) Expression of epiregulin and amphiregulin and K-ras mutation status predict disease control in metastatic colorectal cancer patients treated with cetuximab. J Clin Oncol 25:3230–3237
89. de Reyniès A, Boige V, Milano G et al (2007) KRAS mutation signature in colorectal tumors significantly overlaps with the cetuximab response signature. J Clin Oncol 26:2228–2230
90. Di Fiore F, Blanchard F, Charbonnier F et al (2007) Clinical relevance of KRAS mutation detection in metastatic colorectal cancer treated by cetuximab plus chemotherapy. Br J Cancer 96:1166–1169
91. Wierzbicki R, Jonker DJ, Moore MJ et al (2009) A phase II, multicenter study of cetuximab monotherapy in patients with refractory, metastatic colorectal carcinoma with absent epidermal growth factor receptor immunostaining. Invest New Drugs 23:1803–1810

化学放射線療法にて5年以上Complete Responseが得られた肛門扁平上皮癌の1例

今田 慎也[*1] 大植 雅之[*1] 能浦 真吾[*1] 真貝 竜史[*1] 本告 正明[*1]
岸 健太郎[*1] 宮代 勲[*1] 西山 謹司[*2] 矢野 雅彦[*1] 石川 治[*1]

〔*Jpn J Cancer Chemother* 38(12): 2119-2121, *November*, 2011〕

A Case of Perianal Squamous Cell Carcinoma Attaining a Complete Response Over Five Years with Chemoradiotherapy: Shinya Imada[*1], Masayuki Ohue[*1], Shingo Noura[*1], Tatsushi Shingai[*1], Masaaki Motoori[*1], Kentarou Kishi[*1], Isao Miyashiro[*1], Kinji Nishiyama[*2], Masahiko Yano[*1] and Osamu Ishikawa[*1] ([*1]*Dept. of Gastrointestinal Surgery, and* [*2]*Dept. of Radiation Oncology, Osaka Medical Center for Cancer and Cardiovascular Diseases*)

Summary

We report a case with perianal squamous cell carcinoma, which showed a complete response more than five years after chemoradiotherapy. A 69-year-old-man was introduced to our hospital for the diagnosis of squamous cell carcinoma [T3 (8.0×8.0 cm) N0M0, Stage II]. The patient was treated by chemoradiotherapy, which consisted of 5-FU 750 mg/m^2/day (continuous intravenously) on days 1-5 and 29-33, and mitomycin C 10 mg/m^2 on days 1 and 29 and radiation at 2 Gy/day for 5 days per week (total dose 60 Gy). The patient tolerated this treatment with no severe adverse effects. Tumor disappeared completely 1 month after this treatment with no adjuvant therapy. The patient has been alive with no sign of recurrence for 6 years. **Key words:** Anal carcinoma, Squamous cell carcinoma, Chemoradiotherapy

要旨 肛門扁平上皮癌に対して化学放射線療法を行い，5年以上 complete response が得られた症例を経験したので報告する。症例は60歳台，男性。肛門部痛を主訴に近医を受診，痔瘻切除術の半年後に肛門周囲に腫瘤形成を認め，生検で肛門扁平上皮癌と診断され当院を紹介受診した。受診時，肛門右側に8 cm大の硬結を触知した。肛門扁平上皮癌 T3N0M0，Stage II に対して化学放射線療法を行った。化学療法は5-FU 750 mg/m^2/day の持続静注（days 1～5，29～33）と mitomycin C 10 mg/m^2（day 1，29）の併用とし，放射線療法は総線量60 Gy（2 Gy/day）の多門照射とした。化学放射線療法を終了して1か月後には腫瘍は消失した。化学放射線療法を施行してから6年経過した現在も無再発生存中である。

緒 言

肛門扁平上皮癌に対しては，歴史的に手術療法が行われてきた。しかしながら，1974年にNigroらが化学放射線療法（chemoradiotherapy: CRT）の良好な成績を報告して以降[1]，CRTが手術治療と同等の成績であるとする報告が多くだされ，現在欧米ではCRTが肛門扁平上皮癌（Stage II/III）に対する標準治療として確立されている[2]。一方，本邦では肛門扁平上皮癌はまれであり[3]，肛門扁平上皮癌に対して，CRTは標準治療となっていない。しかしながら鮫島らの報告では，腹会陰式直腸切断術（APR）の施行率は1989年以前の89%に対し1995年以降では49%と低下しており[4]，本邦においてもCRTが施行される頻度は増えてきている。今回われわれは，CRTにより5年以上の長期にわたりcomplete response（CR）が得られた症例を経験したので報告する。

I. 症 例

患者: 60歳台，男性。

主訴: 肛門痛。

既往歴，家族歴: 特記事項なし。

現病歴: 数年前からの肛門部痛を主訴に近医を受診し，肛門周囲膿瘍と診断された。二度の切開排膿術の後に痔瘻切除術を施行したが，その半年後に肛門右側皮膚に潰

[*1] 大阪府立成人病センター・消化器外科
[*2] 同 ・放射線治療科

連絡先: 〒537-8511 大阪市東成区中道1-3-3 大阪府立成人病センター・消化器外科
今田 慎也

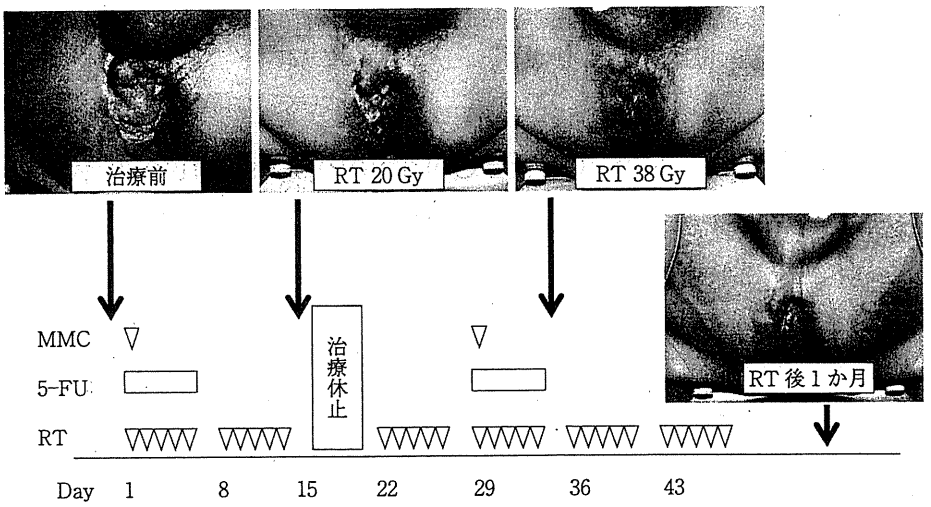

図1 治療経過

瘍および腫瘤を形成したため行った生検で扁平上皮癌と診断され，当院に紹介となった。

受診時現症: 身長 172 cm，体重 65 kg。肛門右側の皮膚に 6×6 cm 大の潰瘍を認め，その周囲に 8×8 cm 大の硬結を触知した。

血液検査所見: 血算，生化学では異常を認めず，腫瘍マーカーは SCC 5.7 ng/mL（正常範囲 1.55 ng/mL 以下），CEA 7.4 ng/mL（正常範囲 5.0 ng/mL 以下）と上昇していた。

治療経過: 肛門扁平上皮癌（cT3N0M0，Stage II），（図1）に対して CRT を開始した[5]。化学療法は 5-FU 750 mg/m^2/day の持続静注を day 1～5 および day 29～33 に，mitomycin C（MMC）10 mg/m^2 を day 1 および day 29 に静注した。放射線療法は多門照射で，右鼠径リンパ節が約 1 cm に腫大し転移が否定できなかったため，右鼠径部を照射野に含め骨盤腔に照射した。治療後 2 週間目に grade 2 の好中球減少を認め，治療を 1 週間休止したが，40 Gy 照射した時点で腫瘍の著明な縮小を認めたため，CRT による CR が期待できると判断し，肛門管・肛門を中心に CRT を 20 Gy 追加した。CRT 終了 1 か月後に，腫瘍は完全に消失した。CRT 後のフォローアップは半年ごとに行ったが，視触診に加えて画像検査（CT，MRI もしくは PET）では 5 年経過した時点で再発徴候はなかった。また，放射線直腸炎などの晩期有害事象を認めず，現在も CR を維持している。

II. 考　察

欧米においては，Stage II/III の肛門扁平上皮癌に対する CRT は標準治療として確立されている[2]。一方，本邦において，Stage II/III の肛門扁平上皮癌に対する CRT は，現時点では標準治療として確立されていない。しかしながら，肛門扁平上皮癌に対して APR が施行される割合は徐々に減少傾向にあり[4]，今後本邦においても CRT の割合が増加することが予想される。

自験例において，化学療法は MMC と 5-FU の併用とした。RTOG 87-04 試験では，MMC/5-FU 併用 CRT と 5-FU 併用 CRT との第 III 相比較試験が行われ[6]，RTOG 98-11 試験では MMC/5-FU 併用 CRT と MMC/CDDP 併用 CRT との第 III 相比較試験が行われた[7]。これらの結果は，いずれも MMC/5-FU 併用 CRT が優れており，欧米では MMC/5-FU が first-line として位置付けられている[7]。しかしながら，MMC を使用した場合には血液毒性の頻度が高いことが報告され，注意が必要である[7]。自験例でも grade 2 の好中球減少を認めた。

放射線量については 60 Gy 前後の線量を推奨している報告が多く[6,8]，自験例も総線量を 60 Gy とした。重篤な有害事象としては，直腸炎，肛門狭窄などが報告されているが[9]，自験例ではこれらの有害事象は認めなかった。

現在本邦では，Stage II/III の肛門扁平上皮癌に対する標準治療を確立すべく，Japan Clinical Oncology Group（JCOG）大腸がんグループにより臨床第 I/II 相試験が行われている。化学療法は S-1 と MMC の併用とし[10]，放射線の総線量は 59.4 Gy としている。当センターにおいても，Stage II/III の肛門扁平上皮癌に対して，現在はこの臨床試験に参加して治療を行っている。

欧米の臨床試験では，肛門扁平上皮癌に対する CRT 施行例の 5 年生存率は 58～94%，無病生存率は 60～80% と報告されている[11]。本邦では，55 例を対象としたアンケート調査で 3 年生存率が 95%，CR 率が 80%，3 年無増悪生存割合が 77% と報告されているものの，観察期間が 2.7 年と短く[3]，CRT 後の長期成績は明らかになっていない。医学中央雑誌で検索した限りでは，本邦で肛門

表1 CRTにより5年以上CRが得られた症例

症例	報告年	著者	年齢	性別	臨床病期	組織型	化学療法	放射線療法（Gy）	転帰
1	2003	石田ら	46	女性	T3N0M0 (Stage II)	mod	5-FU + MMC	66 Gy (外照射 30 Gy + 組織内照射 36 Gy)	5年 無再発
2	2009	栗田ら	63	女性	T2N2M0 (Stage IIIB)	well	5-FU + CDDP	63 Gy（4門照射）	5年3か月 無再発
3	2011	自験例	69	男性	T3N0M0 (Stage II)	well	5-FU + MMC	60 Gy（多門照射）	6年 無再発

扁平上皮癌に対してCRT後に5年以上CRが得られた症例は自験例を含めて3例のみであった（表1）[12,13]。しかしながら，現時点で当センターの5年未満のCR例は4例あり，今後CRT施行例の増加とともに長期CR例は増えていくものと予想される。

結　語

肛門扁平上皮癌に対するCRTは，長期的な予後についても良好な結果が得られる可能性がある。

文　献

1) Nigro ND, Vaitkevicius VK and Considine B Jr: Combined therapy for cancer of anal canal: a preliminary report. *Dis Colon Rectum* **17**(10): 354–356, 1974.
2) NCCN: NCCN Clinical Practice Guidelines in Oncology–v.I 2007. Anal Carcinoma. http://www.nccn.org/. 2007–07–06.
3) Takashima A, Shimada Y, Hamaguchi T, *et al*: Current therapeutic strategies for anal squamous cell carcinoma in Japan. *Int J Clin Oncol* **14**(5): 416–420, 2009.
4) 鮫島伸一，澤田俊夫，長廻　紘：本邦における肛門扁平上皮癌，痔瘻癌の現状，第59回大腸癌研究会アンケート調査報告．日本大腸肛門病会誌　**58**(8): 415–421, 2005.
5) Sobin LH, Gospodarowicz MK and Wittekind C: UICC: TNM classification of Malignant Tumors (7th ed), Wiley–Liss: New York, 2009.
6) Falm M, John M, Pajak TF, *et al*: Role of mitomycin in combination with fluorouracil and radiotherapy, and of salvage chemoradiation in the definitive nonsurgical treatment of epidermoid carcinoma of the anal canal: results of a phase III randomized intergroup study. *J Clin Oncol* **14**(9): 2527–2539, 1996.
7) Ajani JA, Winter KA, Gunderson LL, *et al*: Intergroup RTOG98–11: A phase III randomized study of 5–fluorouracil (5–FU), mitomycin, and radiotherapy versus 5–fluorouracil, cisplatin and radiotherapy in carcinoma of the anal canal. *J Clin Oncol, ASCO Annual Meeting* **24**(18S): abstr 4009, 2006.
8) 唐澤克之，岡本雅彦，伊藤芳紀・他：わが国における肛門癌の根治的（化学）放射線治療成績（全国調査結果）．日癌治療会誌　**42**(2): 265, 2007.
9) 高梨似美，斉藤聖宏，江口真理子・他：肛門扁平上皮癌に対する同時化学放射線療法例の検討．臨放線　**55**(9): 1121–1128, 2010.
10) Boku N, Yamamoto S, Fukuda H, *et al*: Fluorouracil versus combination of irinotecan plus cisplatin versus S–1 in metastatic gastric cancer: a randomised phase 3 study. *Lancet Oncol* **10**(11): 1063–1069, 2009.
11) UKCCCR Anal Cancer Trial Working Party: Epidermoid anal cancer: results from the UKCCCR randomised trial of radiotherapy alone versus radiotherapy, 5–fluorouracil, and mitomycin. *Lancet* **348**(9034): 1049–1054, 1996.
12) 石田秀之，龍田眞行，古河　洋・他：放射線，化学療法が奏効した肛門扁平上皮癌の1例．日外科系連会誌　**28**(6): 1044–1047, 2003.
13) 栗田真紀子，堀江久永，鯉沼広治・他：放射線化学療法により長期にComplete Responseが得られている肛門扁平上皮癌の1例．自治医大紀　**32**: 85–90, 2009.

本論文の要旨は第33回日本癌局所療法研究会において発表した。

Cancer stem cell-related factors are associated with the efficacy of pre-operative chemoradiotherapy for locally advanced rectal cancer

KAZUAKI HIROISHI[1], MASAFUMI INOMATA[1], KENJI KASHIMA[2], KAZUHIRO YASUDA[1], NORIO SHIRAISHI[1], SHIGEO YOKOYAMA[2] and SEIGO KITANO[1]

Departments of [1]Surgery I and [2]Diagnostic Pathology, Oita University Faculty of Medicine, Oita 879-5593, Japan

Received February 2, 2011; Accepted March 18, 2011

DOI: 10.3892/etm.2011.243

Abstract. Pre-operative chemoradiotherapy (CRT) is an important neoadjuvant therapy for locally advanced rectal cancer. In the present study, we investigated the factors that influence the efficacy of pre-operative CRT in locally advanced rectal cancer. We divided 50 patients with locally advanced rectal carcinoma treated with pre-operative CRT into two groups according to the grade of tumor response to pre-operative CRT: low-sensitivity group and high-sensitivity group. As candidates for the prediction of sensitivity to pre-operative CRT, clinicopathological factors and 12 biomarkers, including factors related to tumor growth, cell cycle, apoptosis, tumor stroma and cancer stem cells, were examined immunohistochemically in 48 resected specimens. Thirty-one tumors showed high sensitivity and 19 showed low sensitivity to pre-operative CRT. The status of stem cell-related factors, CD133 and CD24, was significantly associated respectively with sensitivity to pre-operative CRT (P=0.003, P=0.029). In 10 tumors positive for both CD133 and CD24, low sensitivity to CRT was found in 9 (90%), whereas in 16 tumors negative for both CD133 and CD24, low sensitivity was found in 3 (19%). Other pathological parameters were not associated with tumor response to pre-operative CRT. In conclusion, overexpression of cancer stem cell-related factors, CD133 and CD24, is associated with the sensitivity of locally advanced rectal cancer to pre-operative CRT.

Introduction

Colorectal cancer is a leading cause of morbidity and mortality in developed countries (1). In Japan, an increasingly Westernized diet has led to a high incidence of colorectal cancer. Patients with rectal cancers are known to have an increased rate of local recurrence and decreased survival time compared to patients with tumors of the colon, a result due primarily to the surgical constraints imposed by the location of the rectum within the pelvis (2).

Pre-operative chemoradiotherapy (CRT) is a neoadjuvant therapy for locally advanced rectal cancer that reduces the incidence of local recurrence and improves survival (3). Therefore, CRT is widely used in many countries of the world. However, several tumors show a marked response to CRT, whereas others do not. Furthermore, several adverse events related to CRT, such as enteritis, anorexia, cardiac/thromboembolic events, radiation dermatitis and hematologic toxicity, were reported to occur at frequencies of 6-43% (4). Thus, pre-operative indicators of chemoradiosensitivity are required to avoid unnecessary application of pre-operative CRT, yet little is known about potential biological markers that may be associated with response to pre-operative CRT.

Recently, the discovery of rare subpopulations of cancer stem cells has created a new focus in cancer research. The heterogeneity of tumors can be explained by the concept of cancer stem cells supported by anti-apoptotic signaling. There are a few reports on cancer stem cells related to chemoradiation resistance (5,6). Therefore, in this study we investigated the factors, including cancer stem cell-related factors, that influence the sensitivity of locally advanced rectal cancer to pre-operative CRT using surgical resected specimens to consider tumor heterogeneity.

Materials and methods

Patients. A total of 50 patients with locally advanced rectal carcinoma were treated with pre-operative CRT and surgical resection at the Department of Surgery I, Oita University Faculty of Medicine, or associated institutions (Beppu Medical Center, Nakatsu Municipal Hospital, Oita Prefectural Hospital and Nankai Hospital) between January 2000 and May 2010. Tumors were located at the middle or lower third of the rectum and were diagnosed as clinical stage T2, T3 or T4, Nx and M0 (UICC TNM Classification of Malignant Tumours, 2009). T stage was determined by computed tomography (CT) scan or endoscopic ultrasonography. No

Correspondence to: Dr Kazuaki Hiroishi, Department of Surgery I, Oita University Faculty of Medicine, 1-1 Idaigaoka, Hasama-machi, Yufu, Oita 879-5593, Japan
E-mail: m07d5009@med.oita-u.ac.jp

Key words: rectal cancer, pre-operative chemoradiotherapy, cancer stem cell, CD133, CD24

distant metastases were detected on plain chest X-rays or CT scans. Thirty-nine patients were treated with pre-operative CRT and another 11 patients were treated with pre-operative radiotherapy (RT) alone. The total dose of radiation in most cases was 45 Gy within 6 weeks, usually 1.5 Gy per treatment, five times per week. The total dose range was 40-50 Gy. Several chemotherapy regimens were used in the patients treated with CRT: TS-1 (80 mg/m^2) in 21 patients, 5-fluorouracil (5-FU)-based in 5 patients, tegafur/uracil (UFT) and leucovorin or UFT alone in 8 patients, and tegafur in 5 patients. Curative surgery that included total mesorectal excision was performed in all patients after an interval of approximately 4 weeks following completion of pre-operative treatment. Patient informed consent and approval of the local ethics committee was obtained prior to the study.

Immunohistochemistry. A total of 12 biomarkers were chosen as candidate predictive factors for the efficacy of pre-operative CRT (7-13). These factors included tumor growth-related factors, epidermal growth factor receptor (EGFR) and human epidermal growth factor receptor-2 (HER2); cell cycle-related factors, p53, p21, Ki-67 and Bcl-1; apoptosis-related factors, Bcl-2 and apoptosis protease-activating factor-1 (APAF-1); tumor stroma-related factors, vascular endothelial growth factor (VEGF) and macrophage migration inhibitory factor (MIF); and cancer stem cell (tumor initiating cell)-related factors, CD133 and CD24. Postoperative resected specimens were used for immunohistochemistry.

Paraffin-embedded sections of tumor tissue from the resected rectum were cut at a thickness of 4 μm, deparaffinized in xylene and rehydrated. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase for 10 min. For antigen retrieval, sections were autoclaved at 121°C in 10 mM citrate buffer, pH 6.0, for 10 min. Sections were then treated with primary antibodies. Immunostaining was performed by the avidin-biotin-peroxidase complex technique using a Histofine SAB-PO (Multi) kit (Nichirei Co., Tokyo, Japan) and diaminobenzidine for the visualization of the binding antibodies (14). The following primary antibodies were used: EGFR (clone EGFR113, 1:100; Lab Vision Inc., Fremont, CA, USA) (15); p53 (clone DO-7, 1:50; DakoCytomation, Glostrup, Denmark); p21 (clone SX118, 1:40; DakoCytomation); Ki-67 (clone MIB-1, 1:50; DakoCytomation); Bcl-1 (clone SP4; Nichirei Co.) (16); Bcl-2 (clone 124, 1:40; DakoCytomation); APAF-1 (NCL-APAF-1, 1:20; Novocastra, Newcastle, UK) (17); VEGF (VEGF A-20, 1:100; Santa Cruz Biotechnology, Santa Cruz, CA, USA) (18); MIF (FL-115, 1:200; Santa Cruz Biotechnology) (13); CD133 (ab19898, 1:200; Abcam, Tokyo, Japan) (19); and CD24 (clone SN3b, 1:100; Lab Vision Inc.) (20). Immunohistochemistry for HER2 was performed with HercepTest (DakoCytomation) (21). Negative controls were treated identically, omitting the primary antibodies. Tumor positivity for a given marker was evaluated using a pre-determined cut-off of 10% (the average of the percentage of tumor cells stained in five fields at x100 magnification: ≤10% tumor cell staining, negative; >10%, positive) according to previous studies (7,8,22). For Ki-67 immunoreactivity, staining was considered positive at >60% (23). Staining was assessed in the nucleus for p53, p21, Ki-67 and Bcl-1, and in the cytoplasm for EGFR, APAF-1, VEGF, MIF, CD133 and CD24. Immunoreactivity for Bcl-2 and HER2 expression was assessed in both the cytoplasm and/or the cell membrane. Staining intensity was not evaluated.

Table I. Patient and treatment characteristics.

Characteristic	No. of patients (n=50)	%
Age (years)		
Median	64	
Range	40-83	
Gender		
Male	37	74
Female	13	26
Surgery		
Total pelvic exenteration	1	14
Abdominoperineal resection	24	48
Sphincter-preserving operation	19	38
Macropathology		
Circumscribed	41	82
Infiltrative	9	18
Histology[a]		
Well differentiated	9	19
Moderately differentiated	31	66
Poorly differentiated	3	6
Mucinous	4	9
T-category[a]		
pT1	2	4
pT2	8	17
pT3	27	57
pT4	10	21
N-category		
pN0	38	76
pN$^+$	12	24
Vessel invasion		
Negative	25	50
Positive	25	50
Tumor response (CRT sensitivity)		
High sensitivity	31	62
Low sensitivity	19	38

[a]Three tumors were excluded from the pathological study due to complete pathologic tumor regression. CRT, chemoradiotherapy.

Classification of response to pre-operative CRT. Tumor response to pre-operative CRT was evaluated pathologically on postoperative specimens according to the evaluation of the standard of therapeutic effect provided in the General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus edited by the Japanese Society for Cancer of the Colon and Rectum (24). According to these standards, evaluation of the therapeutic effect was categorized according to five grades: grade 0, absence of regressive changes; grade 1a, regressive change of tumor <1/3; grade 1b, regressive change of

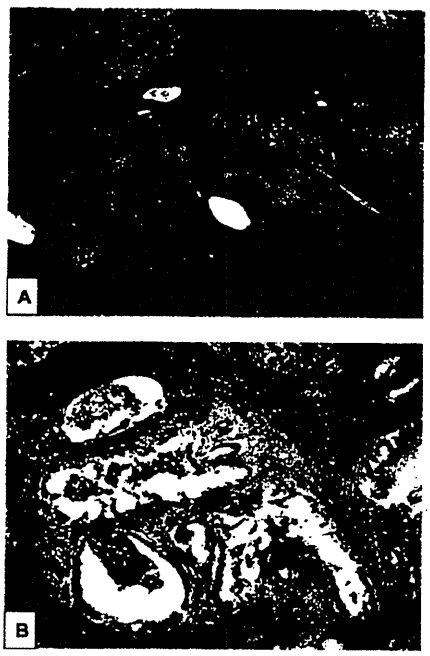

Figure 1. Photomicrographs indicating classification of the pathological response of pre-operative CRT in rectal cancer. (A) High-sensitivity case in which most tumor cells are replaced by fibrosis accompanying the infiltration of inflammatory cells (H&E stain; original magnification, x40). (B) Low-sensitivity case in which most tumor cells remain with mild tumor necrosis and regressive change (H&E stain; original magnification, x40).

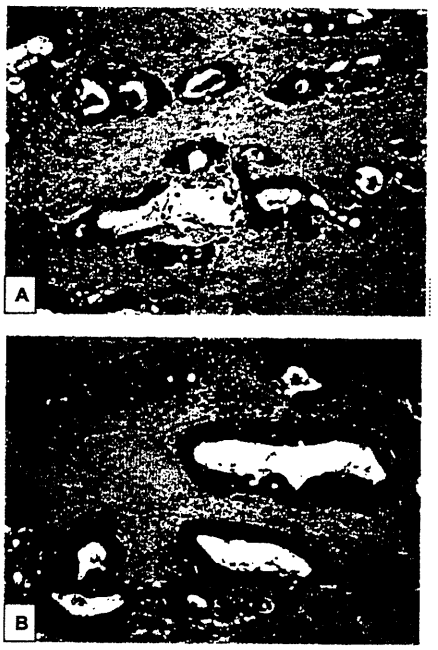

Figure 2. Photomicrographs showing immunohistochemical staining of CD133 and CD24 in rectal carcinoma. (A) Cytoplasmic expression of CD133 in tumor cells is observed (original magnification, x200). Glioblastoma tissue sections were used as a positive control. (B) Strong cytoplasmic expression of CD24 in tumor cells is observed (original magnification, x200). Ovarian serous adenocarcinoma tissue sections were used as a positive control.

tumor <2/3; grade 2, regressive change of tumor >2/3; grade 3, absence of residual tumor cells. We considered grades 0 or 1a to indicate low sensitivity and grades 1b, 2 or 3 to indicate high sensitivity to pre-operative CRT (Fig. 1).

Statistical analysis. For statistical comparisons of patient characteristics between the two groups (low sensitivity and high sensitivity), the Chi-square test, the Fisher's exact probability test or the unpaired t-test was used. A value of $P<0.05$ was considered statistically significant. All analyses were performed with SPSS Software (version 11.0) (SPSS Japan Inc., Tokyo, Japan).

Results

Patient and tumor characteristics. There were 37 (74%) men and 13 (26%) women included in the study. The median age was 64 years (range 40-83). Abdominoperineal resection was performed in 24 (48%) patients and a sphincter-preserving operation was performed in 19 (38%) patients. Macroscopic findings showed 82% of the tumors to be circumscribed tumors and, histologically, most (85%) of the tumors were of the well or moderately differentiated type. Lymph node metastasis was observed in 12 (24%) patients. Vessel invasion was observed in 25 (50%) patients. On the basis of the classification of responses to pre-operative CRT, 31 tumors showed high sensitivity and 19 tumors showed low sensitivity to pre-operative CRT (Table I).

Status of response to CRT according to various clinical parameters. Gender, age, macropathology, location, histology, N-category and surgery were not associated with tumor response (Table II). Of the 10 patients with pT1-2 tumors, 9 showed high sensitivity. The number of pT3-4 tumors showing high sensitivity was nearly equal to those showing low sensitivity ($P=0.034$). Of the tumors negative for vessel invasion, 21 of 25 showed high sensitivity, whereas 15 of 25 tumors positive for vessel invasion showed low sensitivity ($P=0.003$).

Response rates according to various pathological parameters. Factors related to tumor growth, the cell cycle, apoptosis and tumor stroma were not associated with tumor response (Table III). Only factors related to cancer stem cells (tumor-initiating cells) were associated with tumor response. A significant association was found between the resistance of the tumor to treatment and negative CD133 status ($P=0.003$), and there was a significant statistical correlation between the resistance of the tumor to treatment and positive CD24 status ($P=0.029$). In the high-sensitivity tumors, 3 tumors that had complete pathologic tumor regression were excluded from the pathological study (histology and T-category in Tables I and II) and immunohistochemical analysis since the resected specimens did not contain cancer cells (Fig. 2).

Response rates based on combinations of CD133 and CD24. When both CD133 and CD24 were positive, 9 of 10 (90%) tumors showed low sensitivity, whereas when both CD133 and CD24 were negative, 3 of 16 (19%) tumors showed low sensitivity (Table IV). Co-overexpression of CD133 and CD24 was associated with low sensitivity ($CD133^+$ and $CD24^+$ vs. others, $P=0.001$). Negative expression of both CD133 and

Table II. Response according to various clinical parameters.

Parameter	High sensitivity (n=31)	Low sensitivity (n=19)	P-value
Gender			0.481
Male	24	13	
Female	7	6	
Age (years)			0.635
Median	64	65	
Range	44-82	40-83	
Macropathology			0.715
Circumscribed	26	15	
Infiltrative	5	4	
Location			0.273
Upper	4	5	
Lower	27	14	
Histology[a]			0.102
Well/moderate differentiation	26	14	
Poor/mucinous differentiation	2	5	
T-category[a]			0.034
pT1/2	9	1	
pT3/4	19	18	
N-category			0.764
pN0	24	14	
pN1,2	7	5	
Vessel invasion			0.003
Negative	21	4	
Positive	10	15	
Surgery			0.464
LAR/Lap. LAR	13	6	
APR/Lap. APR	18	13	

[a]Three tumors were excluded from pathologic study due to complete pathologic tumor regression. APR, abdominoperineal resection (including total pelvic exenteration); Lap., laparoscopic; LAR, low anterior resection (including sphincter-preserving operation).

CD24 was associated with high sensitivity ($CD133^-$ and $CD24^-$ vs. others, P=0.030).

Discussion

The present study demonstrated that co-overexpression of cancer stem cell-related factors, CD133 and CD24, was significantly associated with locally advanced rectal cancer exhibiting low sensitivity to pre-operative CRT. This result suggests that these two biomarkers may influence sensitivity to pre-operative CRT.

In this study, we used resected specimens from patients who had been treated with pre-operative CRT. For identifying factors which predict the efficacy of CRT before treatment, the use of pre-treatment biopsy specimens is advisable. However, there is heterogeneity in the tumor (5). Therefore, biopsy specimens were not used, and resected specimens were used to investigate the entire tumor specimen.

For the evaluation of CD133 and CD24 expression, immunostaining was classified using the 10% cut-off scoring system. Although one report set the cut-off value to 50%, we adopted the standard system as it has been widely used in many studies. Expression of CD133 and CD24 was distributed evenly within the resected tumors. In the localization of staining, membranous expression of CD24 without cytoplasmic positivity was detected, but we did not include it as being indicative of positive expression.

The concept of cancer stem cells which has been proposed in the field of blood cancer (25) has been adjusted to address solid tumors, such as those of colorectal cancer (26). The fundamental cancer stem cell concept assumes that cancer cells exhibit a hierarchy, as do normal cells, and that a small fraction of cancer cells are maintained as 'cancer stem cells', which have the ability of self-renewal and differentiation (27). Cancer stem cells have recently been proposed to be the cancer-initiating cells that are responsible for tumorigenesis and for contributing to drug resistance in cancer (28). Although a comparatively large number of studies have been reported concerning cancer stem cells and resistance to either chemotherapy or radiotherapy in various cancers, there are few studies available concerning cancer stem cells and resistance to CRT (5).

Table III. Response according to various pathological parameters.

Biomarker	High sensitivity (n=28)	Low sensitivity (n=19)	P-value
HER2			1.000
+	1	0	
-	27	19	
EGFR			0.453
+	4	5	
-	24	14	
VEGF			0.119
+	21	18	
-	7	1	
MIF			0.770
+	13	8	
-	15	11	
p53			0.137
+	24	19	
-	4	0	
p21			0.143
+	5	7	
-	23	12	
Ki-67			0.739
+	19	12	
-	9	7	
Bcl-1			1.000
+	7	4	
-	21	15	
Bcl-2			0.435
+	16	13	
-	12	6	
APAF-1			0.119
+	21	18	
-	7	1	
CD133			0.003
+	2	9	
-	26	10	
CD24			0.029
+	14	16	
-	14	3	

+, positive expression; -, negative expression.

Table IV. Response according to combinations of CD133 and CD24.

Case	High sensitivity (n=28)		Low sensitivity (n=19)	
	No.	%	No.	%
$CD133^+$ and $CD24^{+a}$	1	10	9	90
$CD133^+$ and $CD24^-$	1	100	0	0
$CD133^-$ and $CD24^+$	13	65	7	35
$CD133^-$ and $CD24^{-b}$	13	81	3	19

[a]($CD133^+$ and $CD24^+$) vs. others, P=0.001. [b]($CD133^-$ and $CD24^-$) vs. others, P=0.030.

CD133 and CD24 have been reported as cancer stem cell markers of colorectal cancer in previous studies (26,29,30). CD133 is a 5-transmembrane glycoprotein of 865 amino acids with a total molecular weight of 120 kDa. CD133 antigen expression has been found in such various undifferentiated cells as hematopoietic stem cells (31) and fetal brain stem cells (32). In cancer cells, CD133 has been found to be expressed on cancer stem or tumor-initiating cells in cancers, such as leukemia (33), brain tumors (34) and colorectal cancer. CD24 consists of a small protein core comprising 27 amino acids, which is extensively glycosylated and is bound to the cell membrane via a phosphatidylinositol anchor (35). Several reports have shown that CD24 is expressed in several solid tumors, such as those of small-cell lung cancer and neuroblastoma (36,37), but not in those of colorectal cancer.

Recently, positive clinical studies on the effectiveness of pre-operative CRT on locally advanced rectal cancer have been reported (38). However, pre-operative CRT is not effective in all cases and, actually, cases in which no antineoplastic effect was obtained also exist. Since the treatment period for pre-operative CRT is approximately 10 weeks, patients who obtain no response to CRT lose valuable time during which they could have been treated more effectively. Thus, it is necessary to investigate factors which influence the efficacy of pre-operative CRT.

The results of the present study suggest that the presence of CD133 and CD24 expression is associated with the efficacy of pre-operative CRT. Assuming that CD133 and CD24 are predictive factors of the sensitivity to pre-operative CRT, patients with both $CD133^+$ and $CD24^+$ are expected to have low sensitivity to CRT. So, it may be recommended that such patients undergo surgery without first undergoing CRT. However, since patients with both $CD133^-$ and $CD24^-$ are expected to have high sensitivity to CRT, it may be necessary to aggressively treat these patients first with pre-operative CRT.

In conclusion, the present study shows that the overexpression of cancer stem cell-related factors, CD133 and CD24, is associated with the sensitivity of locally advanced rectal cancer to pre-operative CRT. Further prospective studies are required to establish a new therapeutic system that appropriately uses pre-operative CRT for the benefit of patients with locally advanced rectal cancer. Our group is presently conducting a prospective study using biopsy specimens from pre-therapeutic tumors (UMIN003398). This retrospective study provides valuable information for realization of the ongoing prospective study.

Acknowledgements

The authors thank Dr Koshi Mimori (Department of Surgery and Molecular Oncology, Medical Institute of Bioregulation, Kyushu University); Dr Yoichi Muto, Dr Tetsuya Kusumoto and Dr Toshifumi Matsumoto (Department of Surgery, Beppu

Medical Center); Dr Masahito Ikeda, Dr Fumiaki Kishihara and Dr Akio Shiromizu (Department of Surgery, Nakatsu Municipal Hospital); Dr Hisanobu Sakata and Dr Kyuzo Fujii (Department of Surgery, Oita Prefectural Hospital); Dr Takayuki Kamegawa and Dr Akio Morimoto (Department of Surgery, Nankai Hospital); and Dr Akira Matsumoto (Department of Radiology, Oita University Faculty of Medicine) for their invaluable assistance in this study. They also thank Nagako Katsuki, Mayumi Takeda, Emi Aono and Tomoka Sato for the excellent technical support.

References

1. Jemal A, Siegel R, Ward E, *et al*: Cancer statistics, 2006. CA Cancer J Clin 56: 106-130, 2006.
2. Wolpin BM, Meyerhardt JA, Mamon HJ and Mayer RJ: Adjuvant treatment of colorectal cancer. CA Cancer J Clin 57: 168-185, 2007.
3. Sauer R, Becker H, Hohenberger W, *et al*: Pre-operative versus postoperative chemoradiotherapy for rectal cancer. N Engl J Med 351: 1731-1740, 2004.
4. Calvo FA, Serrano FJ, Diaz-González JA, *et al*: Improved incidence of pT0 downstaged surgical specimens in locally advanced rectal cancer (LARC) treated with induction oxaliplatin plus 5-fluorouracil and pre-operative chemoradiation. Ann Oncol 17: 1103-1110, 2006.
5. Ishii H, Iwatsuki M, Ieta K, *et al*: Cancer stem cells and chemoradiation resistance. Cancer Sci 99: 1871-1877, 2008.
6. Haraguchi N, Inoue H, Tanaka F, *et al*: Cancer stem cells in human gastrointestinal cancers. Hum Cell 19: 24-29, 2006.
7. Zlobec I, Vuong T and Compton CC: The predictive value of apoptosis protease-activating factor 1 in rectal tumors treated with pre-operative, high-dose-rate brachytherapy. Cancer 106: 284-286, 2005.
8. Negri FV, Campanini N, Camisa R, *et al*: Biological predictive factors in rectal cancer treated with pre-operative radiotherapy or radiochemotherapy. Br J Cancer 98: 143-147, 2008.
9. Zlobec I, Vuong T, Compton CC, *et al*: Combined analysis of VEGF and EGFR predicts complete tumour response in rectal cancer treated with pre-operative radiotherapy. Br J Cancer 98: 450-456, 2008.
10. Horst D, Kriegl L, Engel J, Kirchner T and Jung A: CD133 expression is an independent prognostic marker for low survival in colorectal cancer. Br J Cancer 99: 1285-1289, 2008.
11. Haraguchi N, Ohkuma M, Sakashita H, *et al*: $CD133^+CD24^+$ population efficiently enriches colon cancer initiating cells. Ann Surg Oncol 15: 2927-2933, 2008.
12. Choi D, Lee HW, Hur KY, *et al*: Cancer stem cell markers CD133 and CD24 correlate with invasiveness and differentiation in colorectal adenocarcinoma. World J Gastroenterol 15: 2258-2264, 2009.
13. He XX, Chen K, Yang J, *et al*: Macrophage migration inhibitory factor promotes colorectal cancer. Mol Med 15: 1-10, 2009.
14. Tanahashi J, Daa T, Gamachi A, *et al*: Human intestinal spirochetosis in Japan; its incidence, clinicopathologic features, and genotypic identification. Mod Pathol 21: 76-84, 2008.
15. Sato JD, Kawamoto T, Le AD, Mendelsohn J, Polikoff J and Sato GH: Biological effects in vitro of monoclonal antibodies to human epidermal growth factor receptors. Mol Biol Med 1: 511-529, 1983.
16. Cheuk W, Wong KO, Wong CS and Chan JK: Consistent immunostaining for cyclin D1 can be achieved on a routine basis using a newly available rabbit monoclonal antibody. Am J Surg Pathol 28: 801-807, 2004.
17. Paik SS, Jang KS, Song YS, *et al*: Reduced expression of Apaf-1 in colorectal carcinoma correlates with tumor progression and aggressive phenotype. Ann Surg Oncol 14: 3453-3459, 2007.
18. Rosmorduc O, Wendum D, Corpechot C, *et al*: Hepatocellular hypoxia-induced vascular endothelial growth factor expression and angiogenesis in experimental biliary cirrhosis. Am J Pathol 155: 1065-1073, 1999.
19. Dubrovska A, Kim S, Salamone RJ, *et al*: The role of PTEN/Akt/PI3K signaling in the maintenance and viability of prostate cancer stem-like cell populations. Proc Natl Acad Sci USA 106: 268-273, 2009.
20. Kim KH, Choi JS, Kim JM, *et al*: Enhanced CD24 expression in endometrial carcinoma and its expression pattern in normal and hyperplastic endometrium. Histol Histopathol 24: 309-316, 2009.
21. Press MF, Hung G, Godolphin W and Slamon DJ: Sensitivity of HER-2/neu antibodies in archival tissue samples: potential source of error in immunohistochemical studies of oncogene expression. Cancer Res 54: 2771-2777, 1994.
22. Yada K, Shibata K, Matsumoto T, Ohta M, Yokoyama S and Kitano S: Protease-activated receptor-2 regulates cell proliferation and enhances cyclooxygenase-2 mRNA expression in human pancreatic cancer cells. J Surg Oncol 89: 79-85, 2005.
23. Sadahiro S, Suzuki T, Maeda Y, *et al*: Predictors of tumor downsizing and regression with pre-operative radiotherapy alone and with concomitant tegafur/uracil (UFT) for resectable advanced rectal adenocarcinoma. Hepatogastroenterology 54: 1107-1112, 2007.
24. Japanese Society for Cancer of the Colon and Rectum: General Rules for Clinical and Pathological Studies on Cancer of the Colon, Rectum and Anus: The 7th edition, revised version. Tokyo, Kanehara, 2009.
25. Lapidot T, Sirard C, Vormoor J, *et al*: A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. Nature 367: 645-648, 1994.
26. Ricci-Vitiani L, Lombardi DG, Pilozzi E, *et al*: Identification and expansion of human colon-cancer-initiating cells. Nature 445: 111-115, 2007.
27. Reya T, Morrison SJ, Clarke MF and Weissman IL: Stem cells, cancer, and cancer stem cells. Nature 414: 105-111, 2001.
28. Jordan CT, Guzman ML and Noble M: Cancer stem cells. N Engl J Med 355: 1253-1261, 2006.
29. Ieta K, Tanaka F, Haraguchi N, *et al*: Biological and genetic characteristics of tumor-initiating cells in colon cancer. Ann Surg Oncol 15: 638-648, 2008.
30. Weichert W, Denkert C, Burkhardt M, *et al*: Cytoplasmic CD24 expression in colorectal cancer independently correlates with shortened patient survival. Clin Cancer Res 11: 6574-6581, 2005.
31. Yin AH, Miraglia S, Zanjani ED, *et al*: AC133, a novel marker for human hematopoietic stem and progenitor cells. Blood 90: 5002-5012, 1997.
32. Uchida N, Buck DW, He D, *et al*: Direct isolation of human central nervous system stem cells. Proc Natl Acad Sci USA 97: 14720-14725, 2000.
33. Bhatia M: AC133 expression in human stem cells. Leukemia 15: 1685-1688, 2001.
34. Singh SK, Clarke ID, Terasaki M, *et al*: Identification of a cancer stem cell in human brain tumors. Cancer Res 63: 5821-5828, 2003.
35. Fischer GF, Majdic O, Gadd S and Knapp W: Signal transduction in lymphocytic and myeloid cells via CD24, a new member of the phosphoinositol-anchored membrane molecules. J Immunol 144: 638-641, 1990.
36. Jackson D, Waibel R, Weber E, Bell J and Stahel RA: CD24, a signal-transducing molecule expressed on human B cells, is a major surface antigen in small-cell lung carcinomas. Cancer Res 52: 5264-5270, 1992.
37. Akashi T, Shirasawa T and Hirokawa K: Gene expression of CD24 core polypeptide molecule in normal rat tissues and human tumor cell lines. Virchows Arch 425: 399-406, 1994.
38. Roh MS, Colangelo LH, O'Connell MJ, *et al*: Pre-operative multimodality therapy improves disease-free survival in patients with carcinoma of the rectum: NSABP R-03. J Clin Oncol 27: 5124-5130, 2009.