Sensitivity to CPT-11 and platinum derivatives of stage II/ Dukes' B colorectal cancer with occult neoplastic cells in lymph node sinuses

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Abstract. Among 13 patients with recurrent colorectal cancer (recurrence group: RG), immunohistochemical expression of Topo-1 was high in 4 patients (30.8%) and low in 9 patients (69.2%), while the non-recurrence group (N-RG) (n=8) showed high expression in 1 patient (12.5%) and low expression in 7 patients (87.5%) (NS). Regarding immunohistochemical expression of Bax/ERCC-1, high Bax/low ERCC-1 expression was observed in 6 patients (46.2%) from the RG and other patterns of expression were seen in 7 patients (53.8%), while high Bax/low ERCC-1 expression was observed in 4 patients (50.0%) from the N-RG and other patterns were found in 4 patients (50.0%) (NS). PCR analysis of Topo-1 expression revealed high expression in 9 patients (75.0%) from the RG (n=12) and low expression in 3 patients (25.0%), while the N-RG (n=8) showed high expression in all 8 patients (100.0%) and low expression in none (NS). With respect to ERCC-1, PCR analysis revealed high expression in 7 patients (58.3%) from the RG (n=12) and low expression in 5 patients (41.7%), while the N-RG (n=8) showed high expression in 1 patient (12.5%) and low expression in 7 patients (87.5%) ($p<0.05$). These results suggest that tumor sensitivity to CPT-11 and platinum derivatives is similar in stage II colorectal cancer patients with ONCs.

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Abbreviations: ONCs, occult neoplastic cells; LN, lymph node; 5-FU, 5-fluorouracil; LV, leucovorin; CPT-11, irinotecan; Topo-1, topoisomerase-1 inhibitor; L-OHP, oxaliplatin; CDDP, cisplatin; Bax, bcl-2 associated X; ERCC-1, excision repair cross-complementation group 1; TS, thymidylate synthase; DPD, dihydropyrimidine dehydrogenase; ITCs, isolated tumor cells; RG, recurrence group; N-RG, non-recurrence group

Key words: colorectal cancer, occult neoplastic cells, CPT-11, topoisomerase-1 inhibitor, platinum agents, Bax, ERCC-1, isolated tumor cells

Introduction

Tumor metastasis/recurrence in the liver and lungs after curative resection of colorectal cancer is presumed to be related to the survival and proliferation of residual cancer cells that circulate during the perioperative period and escape from the host's immune defenses (1-4). There have been many reports suggesting a close relationship between metastasis/recurrence of cancer and the detection of cytokeratin-positive occult neoplastic cells (ONCs) in lymph node (LN) sinuses distant from the primary tumor (5-8). ONCs are a small number of malignant cells that enter the LNs and can be counted by a simple immunostaining method (patent pending since 2002 in Japan) (2,3). Free cancer cells are mentioned as isolated tumor cells (ITCs) in relation to breast cancer in the sixth version of the TNM classification (9). At the St. Gallen meeting in 2005, ITCs were also defined as cells/clusters with a diameter ≤0.2 mm that had not penetrated the walls of vessels or lymphatics (Viale G, 9th International Conference on Primary Therapy of Early Breast Cancer, St. Gallen, abs. S12, 2005). Larger clusters of floating cancer cells are also observed sometimes, but the significance of such clusters remains unclear. Solitary free ONCs in LN sinuses distant from the primary tumor can be detected by cytokeratin immunostaining, but ONCs are also defined as including clusters of ≤10 cells, while malignant microaggregates contain >10 cells (10). ONC clusters have the potential to cause tumor metastasis/recurrence in any organ, and should be differentiated from micrometastases (0.2-2 mm) anchored in LNs or from ITCs (≤0.2 mm), since ONC clusters seem to be have a higher malignant potential (10-12).

When postoperative adjuvant chemotherapy is performed for stage III colorectal cancer patients with LN metastasis, the combination of irinotecan hydrochloride [CPT-11; a topoisomerase-1 inhibitor (Topo-1) with a novel mechanism of action] with 5-fluorouracil + leucovorin (5-FU/LV) and/or

oxaliplatin (L-OHP; a new-generation platinum analogue) has recently come into widespread use (13,14). The FOLFOX regimen with L-OHP has been reported to be significantly superior to the FOLFIRI regimen for improving the survival of patients with metastatic colorectal cancer, and it has been rapidly adopted in the US and Europe (15,16). Platinum agents such as cisplatin (CDDP) generally inhibit DNA synthesis by forming DNA-platinum adducts. Like 5-FU, their efficacy is largely dependent on the sensitivity or resistance of the target tumor. Pharmacological efficacy has been reported to be related to: a) an increase of DNA excision repair due to the up-regulation of nuclear excision repair genes such as excision repair cross-complementation group-1 (ERCC-1) gene; b) increased expression of anti-apoptotic genes such as those of the Bcl-2 family coupled with up-regulation of the P53 tumor suppressor gene; and c) increased removal of drugs from tumor cells by the CDDP/glutathione (GSH, γ-glutamyl cysteinyl glycine) complex and ATP-binding cassette transporter (ABC transporter) (17-21). Up-regulation of ERCC-1 gene expression is reported to be related to the acquisition of tumor resistance to platinum derivatives such as CDDP and L-OHP (17,19,20). It has also been reported that resistance to apoptosis due to decreased expression of the Bcl-2-associated X (Bax) gene has a marked influence on the acquisition of resistance to platinum derivatives (18). These reports suggest that tumors with high Bax/low ERCC-1 expression may be more sensitive to platinum derivatives.

For assessment of tumor drug sensitivity, immunohistochemical staining of the primary lesion and a molecular biological approach involving the PCR have been tried. However, there have been no reports about immunohistochemical/molecular biological investigation of Topo-1 and Bax/ERCC-1 expression in the primary tumors of patients with stage II colorectal cancer who have ONCs. In the present study, the sensitivity of primary tumors to anti-cancer drugs such as CPT-11 and platinum derivatives was investigated by assessing Topo-1 and Bax/ERCC-1 expression in patients who had stage II/Dukes' B colorectal cancer with ONCs, after which tumor sensitivity was compared between CPT-11 and platinum derivatives.

Patients and methods

A total of 124 patients with stage II/Dukes' B LN-negative primary colorectal cancer underwent curative resection during the 13 years from 1987 to 2001 and met the following criteria: a) complete medical records were available and the presence/absence of recurrence and their survival time could be confirmed; and b) at least 10 LNs without metastasis on routine H&E staining had been harvested during surgery. Of the 124 patients, 21 were registered in the database of the Occult Neoplastic Cells Research and Study Group (President: Dr H. Makuuchi, Tokai University School of Medicine; Group Leader: Dr M. Mukai, Tokai University Hachioji Hospital). In these 21 patients, the presence of ONCs was confirmed by cytokeratin immunohistochemical staining (AE1/AE3; Fuji Chemical Industries, Ltd., Japan) (22-25).

Immunohistochemical staining. The 21 patients were divided into a recurrence group (RG) (n=13) who showed recurrence/metastasis of colorectal cancer within 5 years and a non-recurrence group (N-RG) (n=8) without recurrence after at least 5 years. Immunohistochemical staining of primary tumors for Topo-1 and Bax/ERCC-1 was done by the routine indirect immunoperoxidase method using a monoclonal anti-Topo-1 antibody as the primary antibody (Topo-1; 1D6, Dako, Denmark), as well as polyclonal anti-Bax and anti-ERCC-1 antibodies (Bax; PC66T, Calbiochem, Germany, ERCC-1; SPM243, Spring Bioscience, USA). Minor modifications of the standard method were also applied, such as retrieval of antigenicity (26-29). Stained tumor cells were counted at a high power and each lesion was assigned to one of the following four grades: 0% (-), <5% (+), ≥5% to <30% (++), and ≥30% (+++). Both (-) and (+) were defined as 'low expression', while (++) and (+++) were 'high expression' (Figs. 1 and 2) (22-25). To predict whether or not tumors were likely to be sensitive to CPT-11 and platinum derivatives, the expression of Topo-1, Bax, and ERCC-1 was investigated and the rate of patients with high Topo-1 expression or with high Bax/low ERCC-1 expression was determined.

Real-time PCR. Real-time PCR for detection of Topo-1 and ERCC-1 expression was conducted by the Danenberg tumor profile method using paraffin blocks from the 21 LN-negative cancer patients, who were divided into recurrence and non-recurrence groups. Cut-off values were established to categorize the expression of Topo-1 and ERCC-1 as high or low (30,31).

Laser capture microdissection. A representative formalin-fixed, paraffin-embedded, tumor specimen that was harvested before S1 treatment was selected by a pathologist after examination of the H&E-stained slides. Then sections cut at a thickness of 10 μm and were stained with nuclear fast red to enable visualization of the histology for laser capture microdissection (PALM Microlaser Technologies AG, Bernried, Germany), which was done to ensure that only tumor cells were studied (32-34).

RNA isolation, cDNA synthesis, and reverse transcriptase-PCR. Isolation of RNA from formalin-fixed, paraffin-embedded specimens was done according to the method of Response Genetics, Inc. (Los Angeles, CA; US patent no. 6,248,535). Then, cDNA was prepared from each RNA sample as described previously. Quantitation of cDNAs for Topo-1/ERCC-1 and an internal standard gene (ß-actin) was done using a fluorescence-based real-time detection method [ABI-PRISM 7900 Sequence Detection System (TaqMan), Applied Biosystems, Foster City, CA], as described previously (35,36).

Statistical analysis. The χ^2 test was used to examine the significance of differences between the recurrence and non-recurrence groups and $p<0.05$ was considered to indicate a significant difference. SPSS 13.0 J software (SPSS Japan, Inc., Tokyo, Japan) was used for all analyses.

Results

Immunohistochemistry for Bax/ERCC-1. In the RG (n=13), high Bax expression was observed in 9 patients (69.2%) and

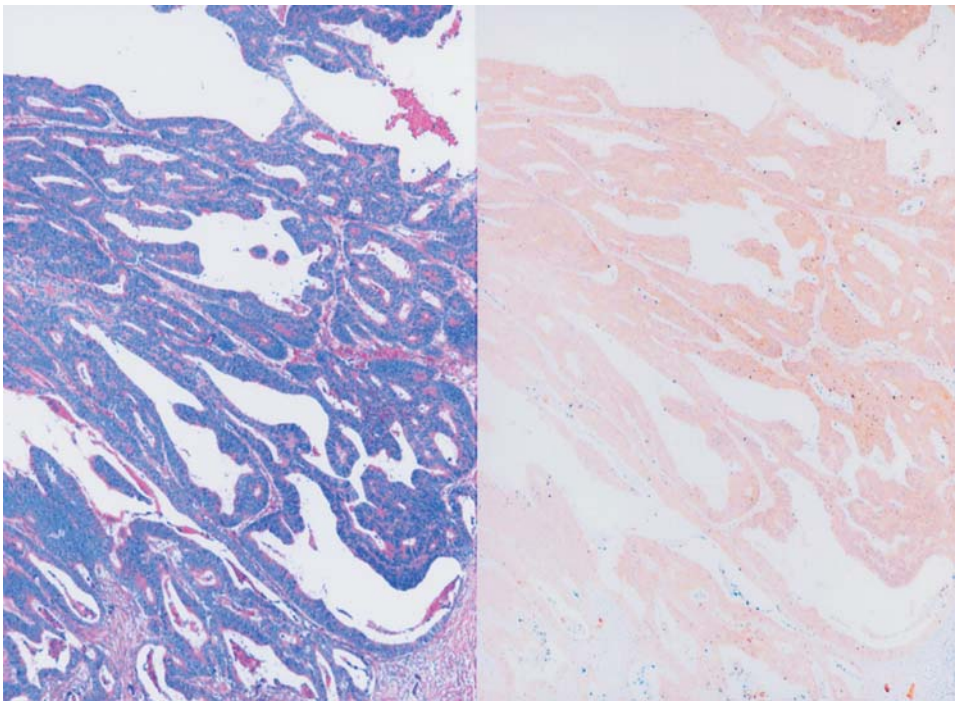

Figure 1. Immunohistochemical staining for topoisomerase-1 inhibitor (Topo-1) (high expression, +++) in the primary tumor of a patient with recurrence of stage II/Dukes' B colorectal cancer and occult neoplastic cells (left: H&E stain, x200; right: Topo-1, x200).

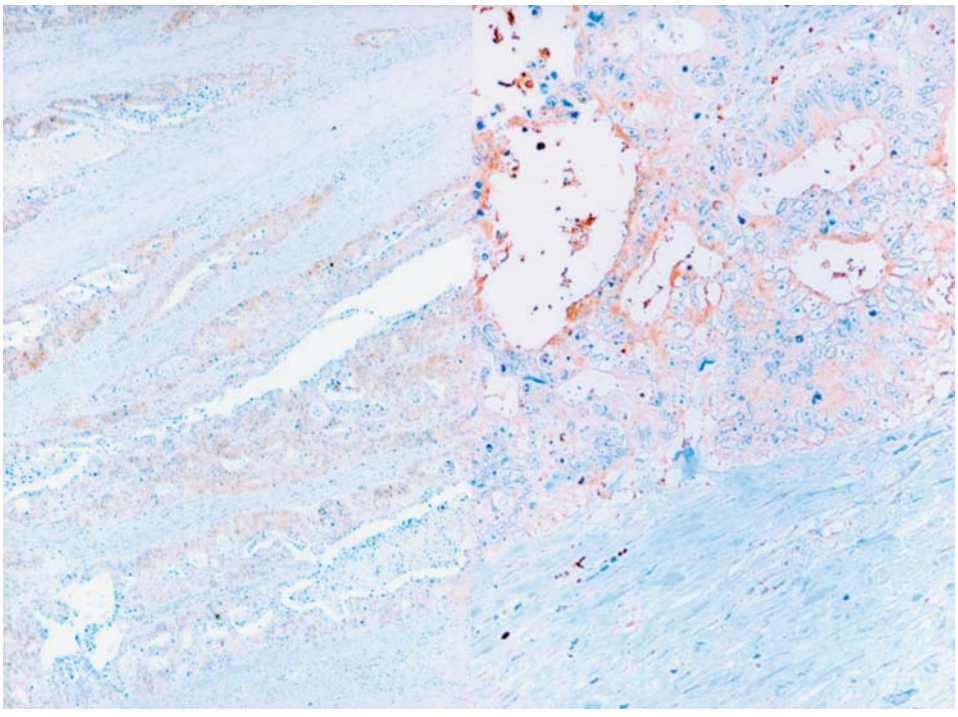

Figure 2. Immunohistochemical staining for Bcl-2-associated X (Bax) and excision repair cross-complementation group 1 (ERCC-1) (high expression, +++) in the primary tumor of a patient with recurrence of stage II/Dukes' B colorectal cancer and occult neoplastic cells (left: Bax, x50; right: ERCC-1, x200).

low expression was found in 4 patients (30.8%). In the N-RG (n=8), high Bax expression was observed in 6 patients (75.0%) and low expression was noted in the other 2 patients (25.0%) (p=not significant; NS) (Table I). In addition, the RG (n=13) showed high ERCC-1 expression in 4 patients (30.8%) and low expression in 9 patients (69.2%), while the N-RG (n=8) showed high expression in 3 patients (37.5%) and low expression in 5 patients (62.5%) (NS) (Table I). There were significant differences of Bax and ERCC-1 expression in the RG ($p<0.05$) (Table I). Otherwise, there were no significant differences of Topo-1 and Bax/ERCC-1 expression between the two groups (Table I).

Table I. Expression of Bax and ERCC-1 in the primary tumors of 21 patients with stage II/Dukes' B colorectal cancer and occult neoplastic cells (ONCs) in their lymph node sinuses.[a]

Primary tumor Total 21 cases	Immunohistochemical staining [high-level, (+++) (++); low-level, (+) (-)] Bax		ERCC-1	
	High-level	Low-level	High-level	Low-level
Recurrence (n=13)	69.2% (9/13)	30.8% (4/13)[b]	30.8% (4/13)	69.2% (9/13)[b]
Non-recurrence (n=8)	75.0% (6/8)	25.0% (2/8)	37.5% (3/8)	62.5% (5/8)

[a]The recurrence group comprised 13 patients and the non-recurrence group contained 8 patients. Expression was classified as low (-, +) or high (++, +++). Bax, Bcl-2 associated X; ERCC-1, excision repair cross-complementation group 1. [b]$p<0.05$.

Table II. Topo-1 expression and high Bax/low ERCC-1 expression in the primary tumors of 21 patients with stage II/Dukes' B colorectal cancer and occult neoplastic cells (ONCs) in their lymph node sinuses.[a]

Primary tumor Total 21 cases	Immunohistochemical staining [high-level, (+++) (++); low-level, (+) (-)] Topo-1		Bax and ERCC-1	
	High-level	Low-level	High Bax/low ERCC-1	Others
Recurrence (n=13)	30.8% (4/13)	69.2% (9/13)	46.2% (6/13)	53.8% (7/13)
Non-recurrence (n=8)	12.5% (1/8)	87.5% (7/8)	50.0% (4/8)	50.0% (4/8)

[a]The recurrence group comprised 13 patients and the non-recurrence group contained 8 patients. Expression was classified as low (-, +) or high (++, +++). Topo-1, topoisomerase-1 inhibitor; Bax, Bcl-2 associated X; ERCC-1, excision repair cross-complementation group 1.

Table III. Expression of Topo-1 and ERCC-1 in the primary tumors of 21 patients with stage II/Dukes' B colorectal cancer and occult neoplastic cells (ONCs) in their lymph node sinuses.[a]

Primary tumor Total 21 cases (undetected, 1 case)	Real-time PCR (low-level < 1.0 ≤ high-level) Topo-1		ERCC-1	
	High-level	Low-level	High-level	Low-level
Recurrence (n=12)	75.0% (9/12)	25.0% (3/12)	58.3% (7/12)	41.7% (5/12)[b]
Non-recurrence (n=8)	100.0% (8/8)	0.0% (0/8)[c]	12.5% (1/8)	87.5% (7/8)[b, c]

[a]The recurrence group included 13 patients and the non-recurrence group had 8 patients. Expression was classified as low (<1.0) or high (≥1.0). Topo-1, topoisomerase-1 inhibitor; ERCC-1, excision repair cross-complementation group 1. [b]$p<0.05$; [c]$p<0.01$.

Immunohistochemistry for Topo-1 and Bax/ERCC-1. In the RG (n=13), high expression of Topo-1 was observed in 4 patients (30.8%) and low expression was seen in 9 patients (69.2%), while the N-RG (n=8) showed high expression in 1 patient (12.5%) and low expression in 7 patients (87.5%) (NS) (Table II). In the RG (n=13), high Bax expression was combined with low ERCC-1 expression (high Bax/low ERCC-1 expression) in 6 patients (46.2%) and other patterns of expression were seen in 7 patients (53.8%), while the N-RG (n=8) showed high Bax/low ERCC-1 expression in 4 patients (50.0%) and other patterns were noted in 4 patients (50.0%) (NS) (Table II). There were no significant differences of Topo-1 and Bax/ERCC-1 expression between the two groups (Table II).

RT-PCR for Topo-1 and ERCC-1. RT-PCR of primary tumor tissue detected Topo-1 and ERCC-1 expression in 20/21 patients (95.2%; 12 from the RG and 8 from the N-RG) (Table III). Expression was within the range of 0.54-3.93 (median 1.19), so the cut-off value was set at 1.0 to separate low from high expression. As a result, the RG (n=12) showed high Topo-1 expression in 9 patients (75.0%) and low

expression in 3 patients (25.0%), while the N-RG group (n=8) showed high Topo-1 expression in all 8 patients (100.0%) and none of them had low expression (NS) (Table III). In the case of ERCC-1, the RG (n=12) showed high expression in 7 patients (58.3%) and low expression in 5 patients (41.7%), while the N-RG (n=8) showed high expression in 1 patient (12.5%) and low expression in 7 patients (87.5%) ($p<0.05$) (Table III). There were significant differences of Topo-1 or ERCC-1 expression in the N-RG ($p<0.01$) (Table III). Otherwise, there were no significant differences of Topo-1 and ERCC-1 expression between the two groups (Table III).

Discussion

There have been many immunohistochemical and molecular biological investigations of resistance to CPT-11 and platinum derivatives using primary tumor tissues, but there has been no previous investigation of Topo-1, Bax, and ERCC-1 expression in the primary tumors of patients with ONCs. We considered that tumor drug sensitivity should be investigated for ONCs which exist in organs/tissues other than the primary lesion and might cause metastasis. However, we thought that it would be difficult to prepare serial sections of LNs for routine H&E staining and then assess Topo-1 and Bax/ERCC-1 expression in the same ONCs, so we studied the primary tumors instead. We found that Topo-1 and Bax/ERCC-1 expression were easily detected because the cancer cells were well stained. In particular, the nucleus and cytoplasm were prominently stained, while the cell membranes, interstitial cells, non-cancerous regions, and normal epithelial cells were weakly stained or unstained. Both CPT-11 and platinum derivatives are DNA synthesis inhibitors, so they were well stained in the nucleus of cancer cells and more strongly stained in the cytoplasm. For further investigation of the localization and distribution of Topo-1 and Bax/ERCC-1, immune electron microscopy may be necessary.

There have been a number of molecular biological investigations of Topo-1 and ERCC-1 in primary tumor tissues, but no previous study has assessed the primary tumors of patients with ONCs. Laser capture microdissection using paraffin blocks requires 10-μm thick sections, and like immunohistochemical investigation, we thought that it would be difficult to assess the staining of the same ONCs, so we studied the primary tumors instead. Expression of Topo-1 and ERCC-1 was detected by PCR in the primary tumors of 20 out of 21 patients (95.2%), with the only exception being 1 patient from the non-recurrence group. Topo-1 expression ranged from 0.85 to 3.93 (median 1.76, mean 1.97), while ERCC-1 expression ranged from 0.54 to 3.46 (median 0.92, mean 1.21). In the present small group of patients, the cut-off value was set at 1.0 for separating low from high expression. However, we need to accumulate data from a larger number of subjects to establish a more accurate cut-off value.

To perform effective postoperative adjuvant chemotherapy for the prevention of metastasis/recurrence after curative resection of primary cancer, it is necessary to eliminate residual tumor cells that circulate through the body during the perioperative period. Treatment with systemic consolidation chemotherapy during the early period after surgery is expected to be most effective for this purpose. According to a report on the sensitivity of tumors to 5-FU + LV, only a few patients had sensitive tumors including those with high TS/low DPD expression at the time when ONC clusters were circulating causing metastasis. This suggests that 5-FU is unlikely to be sufficiently effective (22-25). To solve this problem, CPT-11 and/or L-OHP, which are more widely employed in the US and Europe than in Japan, may be useful as modulators of 5-FU and an additive or synergistic effect can be expected. Although the dosage/administration schedule has an influence on the outcome, sensitivity to the anti-cancer drugs used for combination therapy is presumed to be the key factor determining the results. There was a significant difference in the prevalence of high Bax/low ERCC-1 expression between the recurrence group and the non-recurrence group, so we investigated the number of patients with high Bax/low ERCC-1 expression or high Topo-1 expression in the recurrence group. High Topo-1 expression was observed in 30.8% of the patients, while high Bax/low ERCC-1 expression was seen in 46.2%. More patients had high Topo-1 expression in the recurrence group than in the non-recurrence group (12.5%). PCR analysis revealed high Topo-1 expression in 75.0% and low ERCC-1 expression was seen in 41.7%, but only a small number of patients was studied and no significant differences were observed. However, both our immunohistochemical and PCR findings suggested that it may be better to use CPT-11 as a first-line drug for postoperative adjuvant combination therapy in high-risk patients with stage II colorectal cancer and ONCs. The cost of L-OHP therapy is covered by government health insurance in Japan if it is used for the treatment of metastasis/recurrence in organs such as the liver or lungs, so it may be reasonable to try L-OHP if adjuvant therapy with CPT-11 is not sufficiently effective.

Improvement of the overall survival rate after recurrence is considered to be most important for improving the prognosis (37,38). For the treatment of high-risk patients with ONC-positive stage II colorectal cancer, it appears to be important to detect such patients soon after curative resection and provide appropriate treatment at the stage of occult systemic metastasis before overt recurrence occurs. For sufficient efficacy, it also appears to be preferable to use 5-FU and other agents as combination therapy. It is possible that the standard multidrug adjuvant chemotherapy for stage III cancer patients may also be effective for patients with stage II cancer. However, it will be necessary to investigate more patients in order to determine whether intravenous drug therapy is necessary for patients with stage II/Dukes' B colorectal cancer and ONCs.

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References

1. Mukai M, Ito I, Mukoyama S, Tajima T, Saito Y, Nakasaki H, Sato S and Makuuchi H: Improvement of 10-year survival by Japanese radical lymph node dissection in patients with Dukes' B and C colorectal cancer: a 17-year retrospective study. Oncol Rep 10: 927-934, 2003.

2. Mukai M, Sato S, Komatsu N, Nishida T, Shiba K, Ito I, Nakasaki H and Makuuchi H: Correlation between occult neoplastic cells in the lymph node sinuses and recurrence in patients with Dukes' C colorectal cancer. Oncol Rep 10: 1165-1169, 2003.
3. Mukai M, Sato S, Komatsu N, Nishida T, Shiba K, Ito I, Nakasaki H and Makuuchi H: Correlation between occult neoplastic cells in the lymph node sinuses and recurrence in patients with curatively resected Dukes' B colorectal cancer. Oncol Rep 10: 1177-1181, 2003.
4. Mukai M, Sato S, Nishida T, Komatsu N, Shiba K, Nakasaki H and Makuuchi H: Selection criteria for high risk and low risk groups of recurrence and metastasis in patients with primary colorectal cancer. Oncol Rep 10: 1753-1758, 2003.
5. Mukai M, Sato S, Nakasaki H, Saito Y, Nishiumi N, Iwasaki M, Tokuda Y, Ogoshi K, Inoue H and Makuuchi H: Occult neoplastic cells in the lymph node sinuses and recurrence of primary breast, lung, esophageal and gastric cancer. Oncol Rep 11: 81-84, 2004.
6. Mukai M, Sato S, Tajima T, Kimura T, Komatsu N, Ninomiya H, Nakasaki H, Ogoshi K and Makuuchi H: Predicting recurrence and metastasis of stage II/Dukes' B colorectal cancer without lymph node metastasis. Oncol Rep 12: 1127-1130, 2004.
7. Mukai M, Sato S, Tajima T, Kimura T, Komatsu N, Ninomiya H, Nakasaki H, Ogoshi K and Makuuchi H: Predicting recurrence and metastasis of stage III/Dukes' C colorectal cancer with lymph node metastasis. Oncol Rep 12: 1301-1304, 2004.
8. Mukai M, Sato S, Tajima T, Kimura T, Komatsu N, Ninomiya H, Nakasaki H, Ogoshi K and Makuuchi H: Predicting recurrence and metastasis of Dukes' A primary colorectal cancer with or without proper muscle invasion. Oncol Rep 12: 1305-1308, 2004.
9. TNM Classification of Malignant Tumours. 6th edition, John Wiley & Sons, Inc., New York, 2002.
10. Mukai M: Occult neoplastic cells and malignant micro-aggregates in lymph node sinuses: review and hypothesis. Oncol Rep 14: 173-175, 2005.
11. Mukai M, Sato S, Ninomiya H, Wakui K, Komatsu N, Tsuchiya K, Tajima T, Nakasaki H and Makuuchi H: Prospective study on the recurrence/metastasis of stage II/III colorectal and gastric cancer associated with occult neoplastic cells in lymph node sinuses: three-year interim results. Oncol Rep 16: 405-410, 2006.
12. Mukai M, Oida Y, Tajima T, Kishima K, Ninomiya H, Sato S, Nakamura M, Nakasaki H and Makuuchi H: Alternating hepatic arterial infusion and systemic chemotherapy for stage IV colorectal cancer with synchronous liver metastasis. Oncol Rep 16: 865-870, 2006.
13. Douillard JY, Cunningham D, Roth AD, Navarro M, James RD, Karasek P, Jandik P, Iveson T, Carmichael J, Alaki M, Gruia G, Awad L and Rougier P: Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomized trial. Lancet 355: 1041-1047, 2000.
14. Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirotta N, Elfring GL and Miller L: Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. Irinotecan Study Group. N Engl J Med 343: 905-914, 2000.
15. Tournigand C, Achille TAE, Lledo G, Flesh M, Quinaux DM-ME, Couteau C, Buyse M, Ganem G, Landi B, Colin P, Louvet C and De Gramont A: FOLFIRI followed by FOLFOX 6 or the reverse sequence in advanced colorectal cancer: a randomized GERCOR study. J Clin Oncol 22: 229-237, 2004.
16. Colucci G, Gebbia V, Paoletti G, Giuliani F, Caruso M, Gebbia N, Cartenì G, Agostara B, Pezzella G, Manzione L, Borsellino N, Misino A, Romito S, Durini E, Cordio S, Seri MD, Lopez M and Maiello E: Phase III randomized trial of FOLFIRI versus FOLFOX 4 in the treatment of advanced colorectal cancer: a multicenter study of Gruppo Oncologico Dell'Italia Meridionale. J Clin Oncol 23: 4866-4875, 2005.
17. Dabholkar M, Vionnet J, Bostick-Bruton F, Yu JJ and Reed E: Messenger RNA levels of XPAC and ERCC1 in ovarian cancer tissue correlate with response to platinum-based chemotherapy. J Clin Invest 94: 703-708, 1994.
18. Perego P, Giarosa M, Righetti SC, Supino R, Caserini C, Delia D, Pierotti MA, Miyashita T, Reed JC and Zunino F: Association between cisplatin resistance and mutation of p53 gene and reduced bax expression in ovarian carcinoma cell systems. Cancer Res 56: 556-562, 1996.
19. Li Q, Gardner K, Zhang L, Tsang B, Bostick-Bruton F and Reed E: Cisplatin induction of ERCC-1 mRNA expression in A2780/CP70 human ovarian cancer cells. J Biol Chem 273: 23419-23425, 1998.
20. Hector S, Bolanowska-Higdon W, Zdanowicz J, Hitt S and Pendyala L: *In vitro* studies on the mechanisms of oxaliplatin resistance. Cancer Chemother Pharmocol 48: 398-406, 2001.
21. Arnould S, Hennebelle I, Canal P, Bugat R and Guichard S: Cellular determinants of oxaliplatin sensitivity in colon cancer cell lines. Eur J Cancer 39: 112-119, 2003.
22. Mukai M, Sato S, Ninomiya H, Wakui K, Komatsu N, Tsuchiya K, Nakasaki H and Makuuchi H: Recurrence and 5-FU sensitivity of stage III/Dukes' C colorectal cancer with occult neoplastic cells in lymph node sinuses. Oncol Rep 14: 1165-1169, 2005.
23. Mukai M, Sato S, Ninomiya H, Wakui K, Komatsu N, Tsuchiya K, Nakasaki H and Makuuchi H: Recurrence and 5-FU sensitivity of stage II/Dukes' B colorectal cancer with occult neoplastic cells in lymph node sinuses. Oncol Rep 14: 1171-1176, 2005.
24. Mukai M, Sato S, Ninomiya H, Wakui K, Komatsu N, Tsuchiya K, Nakasaki H and Makuuchi H: Recurrence and 5-FU sensitivity of node-positive stage II/III gastric cancer with occult neoplastic cells in lymph node sinuses. Oncol Rep 14: 1505-1510, 2005.
25. Mukai M, Sato S, Ninomiya H, Wakui K, Komatsu N, Tsuchiya K, Nakasaki H and Makuuchi H: Recurrence and 5-FU sensitivity of node-negative stage I/II breast, lung, or gastric cancer with occult neoplastic cells in lymph node sinuses. Oncol Rep 15: 815-820, 2006.
26. Nakane PK and Pierce GB: Enzyme-labeled antibodies: preparation and application for localization of antigens. J Histochem Cytochem 14: 929-931, 1966.
27. Nakane PK and Pierce GB: Enzyme-labeled antibody for the light and electron microscopic localization of tissue antigens. J Cell Biol 33: 307-318, 1967.
28. Okabe H, Tsujimoto H and Fukushima M: Preparation of the antibodies against recombinant human thymidylate synthase for the detection of its intratumoral levels and application to sensitivity-study of 5-fluorouracil. Oncol Rep 4: 685-690, 1997.
29. Kamoshida S, Shiogama K, Matsuoka H, Matsuyama A, Shimomura R, Inada K, Maruta M and Tsutsumi Y: Immunohistochemical demonstration of dihydropyrimidine dehydrogenase in normal and cancerous tissues. Acta Histochem Cytochem 36: 471-479, 2003.
30. Heid CA, Stevens J, Livak KJ and Williams PM: Real-time quantitative PCR. Genome Res 6: 986-994, 1996.
31. Gibson UE, Heid CA and Williams PM: A novel method for real time quantitative RT-PCR. Genome Res 6: 995-1001, 1996.
32. Lord RV, Salong D, Danenberg KD, Peter JH, De Meester SR, Bremner CG, Tsai PI and Danenberg PV: Telomerase reverse transcriptase expression is increased early in the Barrett's metaplasia, dysplasia, adenocarcinoma sequence. J Gastrointest Surg 4: 135-142, 2000.
33. Uchida K, Hayashi K, Kawakami K, Schneider S, Yochim JM, Kuramochi H, Takasaki K, Danenberg KD and Danenberg PV: Loss of heterozygosity at the thymidylate synthase (TS) locus on chromosome 18 affects tumor response and survival in individuals heterozygous for a 28-bp polymorphism in TS gene. Clin Cancer Res 10: 433-439, 2004.
34. Uchida K, Schneider S, Yochim JM, Kuramochi H, Hayashi K, Takasaki K, Yang D, Danenberg KD and Danenberg PV: Intratumoral COX-2 gene expression is a predictive factor for colorectal cancer response to fluoropyrimidine-based chemotherapy. Clin Cancer Res 11: 3363-3368, 2005.
35. Shirota Y, Stoehlmacher J, Brabender J, Xiong Y-P, Uetake H, Danenberg KD, Groshen S, Tsao-Wei DD, Danenberg PV and Lenz H-J: ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. J Clin Oncol 19: 4298-4304, 2001.
36. Vallböhmer D, Iqbal S, Yang DY, Rhodes KE, Zhang W, Gordon M, Fazzone W, Schultheis AM, Sherrod AE, Danenberg KD and Lenz H-J: Molecular determinants of irinotecan efficacy. Int J Cancer 119: 2435-2442, 2006.
37. Feinstein AR, Sosin DM and Wells CK: The Will Rogers phenomenon: stage migration and new techniques as a source of misleading statistics for survival in cancer. N Engl J Med 312: 1604-1608, 1985.
38. Bunt AMG, Hermans J, Smit VTHBM, van de Velde CJH, Fleuren GL and Bruijn JA: Surgical/pathologic stage migration confounds comparisons of gastric cancer survival rates between Japan and Western countries. J Clin Oncol 13: 19-25, 1995.