

Usefulness of Circulating Tumor Cells after Preliminary Chemotherapy for Prediction of Response to Further Anticancer Therapy in Patients with Initially Unresectable Metastatic Colorectal Cancer

KAI NEKI¹, HIDEJIRO KAWAHARA¹, KAZUHIRO WATANABE¹, YOICHI TOYAMA¹,
TADASHI AKIBA¹ and KATSUHIKO YANAGA²

¹Department of Surgery, Kashiwa Hospital, Jikei University School of Medicine, Chiba, Japan;

²Department of Surgery, Jikei University School of Medicine, Tokyo, Japan

Abstract. *Background/Aim:* The aim of this study was to evaluate the usefulness of circulating tumor cells (CTCs) after preliminary chemotherapy for prediction of response to further anticancer therapy in patients with initially unresectable metastatic colorectal cancer. *Patients and Methods:* CTCs from 14 consecutive patients with Kirsten rat sarcoma viral oncogene homolog (KRAS) wild-type colorectal cancer with synchronous or metachronous unresectable metastatic lesions were measured using the CellSearch system between January 2009 and December 2011. CTCs were measured before and after chemotherapy. The regimen consisted of four courses (three months) of oxaliplatin with oral S-1 (SOX) + panitumumab. *Results:* Ten (71%) out of all patients had no detectable CTCs after chemotherapy. Eight out of these ten patients received further chemotherapy, and liver metastases were completely resected in the other two patients; none of these patients died of cancer within a year after starting chemotherapy. The remaining four patients with CTCs continued to have CTCs after chemotherapy, and all four of these patients died of cancer within eight months after starting chemotherapy. The prognosis of the patients who had no detectable CTCs after the chemotherapy was significantly better than that of those who had CTCs even after the chemotherapy ($p < 0.01$). *Conclusion:* CTCs after preliminary chemotherapy may be useful in predicting the response to further anticancer therapy.

Patients with initially unresectable metastatic colorectal cancer may conceptually be divided into three groups. In the first group, initially unresectable metastatic lesions may shrink remarkably and can then undergo resection after chemotherapy with fair outcome (1-3). In the second group, the patients receive continuous chemotherapy without resection and with improved survival, which is called 'palliative chemotherapy' (4-7). The third group consists of those whose disease progresses even after chemotherapy and further chemotherapy is abandoned. When the diagnosis of initially unresectable metastatic colorectal cancer is entertained, it is actually difficult to classify patients into these three groups. The aim of this study was to evaluate the usefulness of determining circulating tumor cells (CTCs) after preliminary chemotherapy in predicting outcome in patients with unresectable metastases from colorectal cancer.

Patients and Methods

Patient eligibility. The protocol was approved by the Ethics Committee for Biomedical Research of the Jikei Institutional Review Board 21-010 (5588) for a 3-year period between January 2009 and December 2011, and all patients provided their written informed consent. This prospective study was conducted in patients whose eligibility required compliance with the following criteria: Kirsten rat sarcoma viral oncogene homolog (KRAS) wild-type colorectal adenocarcinoma with synchronous or metachronous unresectable metastatic lesions, such as liver metastases, who underwent curative resection of the primary lesion, so-called R0 operation, as defined in the Japanese Classification of Colorectal Carcinoma (8); sufficient oral intake; no prior treatment except for surgery; and age between 30 and 80 years. Patients also had to have adequate organ function ($4,000 \leq \text{leukocytes} < 12,000/\text{mm}^3$; thrombocytes, $\geq 100,000/\text{mm}^3$; serum total bilirubin, $\leq 1.5 \text{ mg/dl}$; aspartate aminotransferase (AST) and alanine aminotransferase (ALT), $< 100 \text{ IU/l}$; and creatinine, $\leq 1.5 \text{ mg/dl}$). Patients were expected to receive medication and to be followed-up regularly for more than 24 weeks. Patients with a history

Correspondence to: Hidejiro Kawahara, MD, Ph.D., Department of Surgery, Kashiwa Hospital, Jikei University School of Medicine, 163-1 Kashiwashita, Kashiwashi, Chiba 277-8567, Japan. Tel: +81471641111 Ext. 3421, Fax: +81 471633488, e-mail: kawahide@jikei.ac.jp

Key Words: Colorectal cancer, circulating tumor cell, prediction of progression.

Table I. *Clinicopathological features of the patients included in this study.*

Variable	No. of patients (%)
Gender	
Male	12 (86)
Female	2 (17)
Age, years (range)	65.8 (56-78)
Number of metastatic sites	
1	7 (50)
2	6 (43)
≥3	1 (7)
Previous chemotherapy	
No	9 (64)
Yes	5 (36)
Serum CEA	
<50 ng/ml	2 (14)
≥50 ng/ml	12 (86)
Circulating tumor cells (/7.5ml)	
Negative	5 (36)
Positive	9 (64)

CEA: Carcinoembryonic antigen.

of drug hypersensitivity, serious surgical and non-surgical complications, or active secondary cancer were excluded. Pregnant or lactating women were also excluded.

Treatment schedule. Physical examination, routine blood analysis, serum carcinoembryogenic antigen (CEA) measurement, computed tomography (CT) and CTC measurement were counted out before and after chemotherapy. CTCs were counted by the CellSearch system (Veridex, Raritan, NJ, USA).

The chemotherapy regimen consisted of four courses (three months) of oxaliplatin with oral S-1 (tegafur, gineracil, oteracil potassium; Taiho Pharmaceutical, Tokyo, Japan) plus panitumumab. The regimen of one course of chemotherapy consisted of oxaliplatin at 130 mg/m² and panitumumab at 6 mg/kg administered on the first day respectively, followed by 14-day administration and six days withdrawal of S-1 at 80 mg or 100 mg per day according to the body surface area (BSA): 80 mg/day for BSA<1.5m²; and 100 mg/day for BSA>1.5m². S-1 was administered orally twice daily after meals.

Isolation of CTCs. Blood samples were drawn into 10-ml evacuated tubes (CellSave; Immunicon, Huntingdon Valley, PA, USA). Samples were maintained at room temperature and processed within 72 h after collection. The determination of CTCs in blood was performed with the semi-automated CellSearch System (Veridex). CTCs were defined as epithelial cell adhesion molecule (EpCAM) isolated intact cells staining positively for cytokeratin and negatively for CD45, and we judged CTC-negative cases to be those with fewer than three CTCs per sample.

Statistical analysis. All data were analyzed using the Statistical Package for Social Sciences (SPSS 18). The statistical significance was determined by the Mann-Whitney *U*-test and the Chi-square test. The survival rate was calculated by the Kaplan Meier method, and the statistical significance was determined by the generalized Wilcoxon test. A *p*-value of less than 0.05 indicates statistical significance.

Table II. *Response to chemotherapy in the patients who received chemotherapy.*

	Previous chemotherapy	
	No (n=9)	Yes (n=5)
Courses of chemotherapy		
<4	0	2
≥4	9	3
Conversion therapy	2	0
Serum CEA (ng/ml)		
Before chemotherapy		
<50	2	0
≥50	7	5
After chemotherapy		
<50	7	1
≥50	2	4
CTCs (/7.5 ml)		
Before chemotherapy		
0-2	5	0
3-39	4	2
≥40	0	3
After chemotherapy		
0-2	9	1
3-39	0	1
≥40	0	3

CEA: Carcinoembryonic antigen; CTCs: circulating tumor cells.

Results

Characteristics of the patients (Table I). Between January 2009 and December 2011, 14 patients were enrolled into the study. Their characteristics are summarized in Table I. Nine patients (64%) received the chemotherapy as first-line therapy, and the other five patients (36%) received the regimen as the third line. Serum CEA levels of twelve patients (86%) were more than 50 ng/ml, which was ten times as high as the normal limit. Five patients (36%) had no CTCs before chemotherapy, even though they had unresectable metastatic lesions.

Response to chemotherapy in the patients who received chemotherapy (Table II). Nine patients had received no previous chemotherapy and were able to tolerate four course of chemotherapy according to the regimen. Of these, two patients underwent hepatic resection as a conversion therapy. Two out of the other five patients who had undergone previous chemotherapy were unable to tolerate four courses of chemotherapy because of the deterioration of their general condition due to the chemotherapy.

Only two out of the nine patients who received no previous chemotherapy had serum CEA levels of less than 50 ng/ml before chemotherapy. After chemotherapy, serum CEA levels of another five out of the nine decreased to less than 50 ng/ml. On the other hand, none of the five patients, who received

Table III. Clinicopathological features of the patients with and without circulating tumor cells (CTCs) after chemotherapy.

	CTCs after chemotherapy		p-Value
	No (n=10)	Yes (n=4)	
Gender			
Male	10	2	0.066
Female	0	2	
Age, years	67.4 (56-78)*	61.8 (58-65)*	0.199
Number of metastatic sites	1.4 (1-2)*	2 (1-3)*	0.155
Previous chemotherapy			
No	9	0	0.005
Yes	1	4	
Serum CEA (ng/ml)			
Before chemotherapy	113.0 (16.0-425.7)*	3,928.4 (240.0-14661.0)*	0.011
After chemotherapy	32.5 (4.6-95.0)*	11,527.5 (450.8-31745.0)*	0.002
Outcome (at 6 months)			
Alive	10	1	0.011
Dead	0	3	

*Range; CEA: Carcinoembryonic antigen; CTCs: circulating tumor cells.

previous chemotherapy had a serum CEA level of less than 50 ng/ml before the chemotherapy, while only in one patient did the serum CEA level decrease to less than 50 ng/ml after the chemotherapy.

Five out of the nine patients who received no previous chemotherapy had no detectable CTCs before chemotherapy, while none of the nine patients had detectable CTCs after the chemotherapy. On the other hand, CTCs were detectable in all five patients who received previous chemotherapy before the chemotherapy, while only one out of the five had no detectable CTCs after the chemotherapy.

Comparison between CTC-negative and -positive patients after the chemotherapy (Table III). Ten patients who had no CTCs after chemotherapy had a significantly lower serum CEA level than the other four patients with CTCs both before and after chemotherapy. Furthermore, all ten patients without CTCs after chemotherapy were alive over a year after starting chemotherapy. On the other hand, all four patients with CTCs even after the chemotherapy died of cancer within eight months after starting chemotherapy. In particular, three patients increase in CTCs after chemotherapy died of cancer within six months after starting chemotherapy.

The survival period of the patients who had no detectable CTCs after the chemotherapy was significantly better than that of the patients who had CTCs even after the chemotherapy ($p < 0.01$) (Figure 1).

Discussion

CTCs can be released from the primary tumor and metastatic lesions into the bloodstream. In early stages, the immune surveillance system eliminates such cells from the circulation,

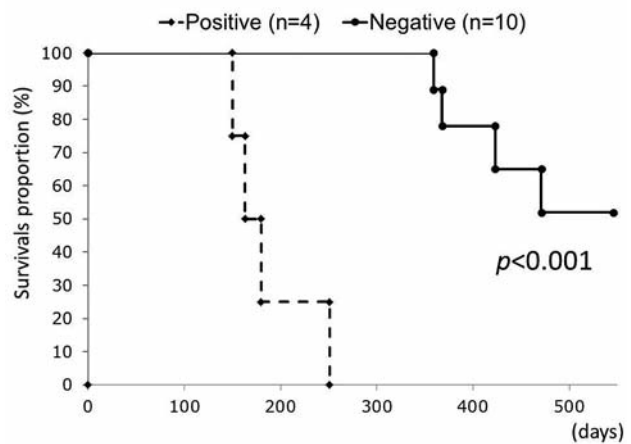


Figure 1. Comparison of survival between patients with and without circulating tumor cells (CTCs) after preliminary chemotherapy. The survival of the patients without detectable CTCs after chemotherapy was significantly better than that of those with CTCs even after chemotherapy ($p < 0.01$).

but CTCs may persist longer and be detected later. Recent studies have highlighted the prognostic significance of the presence and number of CTCs (9-12). CTCs in the peripheral blood have been reported as a prognostic factor in colorectal cancer (9-12). A decrease in CTCs after anticancer treatment may be associated with disease remission (11). CTCs and response to chemotherapy in patients with unresectable metastases from colorectal cancer may have a strong relationship (13). Thus, we tried to evaluate the usefulness of the CTC count after preliminary chemotherapy for initially unresectable metastatic colorectal cancer to predict the response to further anticancer therapy.

Ten out of all patients had no detectable CTCs after the chemotherapy, and eight of these received further chemotherapy while liver metastases were completely resected in the other two patients. On the contrary, the other four patients continued to have CTCs after the chemotherapy and died of cancer within eight months after starting chemotherapy. The prognosis of the patients who had no detectable CTCs after the chemotherapy was significantly better than that of the patients who had CTCs even after the chemotherapy. Although the combined use of 5-FU/leucovorin, as well as the addition of irinotecan or oxaliplatin (FOLFIRI, FOLFOX) (4, 5) to therapy, has increased the median survival time to over 20 months for patients with unresectable metastases from colorectal cancer, the effect of such chemotherapy was not found for cases with advanced metastases.

A method by which patients with unresectable metastases are distinguished into responders and non-responders to chemotherapy is necessary. Our preliminary chemotherapy including the molecular targeted drug panitumumab may be useful in predicting the response to further chemotherapy in patients with unresectable metastatic colorectal cancer. The patients who had CTCs even after preliminary chemotherapy may benefit from best supportive care rather than further chemotherapy. Avoidance of unnecessary chemotherapy is important both for patients indicated best supportive care and in terms of medical economy.

In the present study, two out of ten patients having no detectable CTCs after the chemotherapy underwent hepatectomy for initially unresectable liver metastases. When marked tumor shrinkage is obtained by systemic chemotherapy, initially unresectable metastatic lesions may become resectable. This is called 'conversion therapy'. Adam *et al.* (1) reported that 13% of unresectable liver metastases became resectable following chemotherapy and that the 5-year survival rate after resection in these cases was 33%. A favorable long-term prognosis can thus be expected by conversion therapy.

In conclusion, CTCs after preliminary chemotherapy may be useful in predicting response to further chemotherapy and surgical treatment in patients with unresectable metastases from colorectal cancer.

Conflicts of Interest

We declare that we have no conflict of interests.

References

- Adam R, Delvart V, Pascal G, Valeanu A, Castaing D, Azoulay D, Giacchetti S, Paule B, Kunstlinger F, Ghémard O, Levi F and Bismuth H: Rescue surgery for unresectable colorectal liver metastases downstaged by chemotherapy: A model to predict long-term survival. *Ann Surg* 240: 644-657, 2004.
- Nuzzo G, Giulianti F, Ardito F, Vellone M, Pozzo C, Cassano A, Giovannini I and Barone C: Liver resection for primarily unresectable colorectal metastases downsized by chemotherapy. *J Gastrointest Surg* 11: 318-324, 2007.
- Sugihara K and Uetake H: Therapeutic strategies for hepatic metastasis of colorectal cancer: overview. *J Hepatobiliary Pancreat Sci* 19: 523-527, 2012.
- Cassidy J, Clarke S, Díaz-Rubio E, Scheithauer W, Figer A, Wong R, Koski S, Rittweger K, Giberg F and Saltz L: XELOX vs. FOLFOX-4 as first-line therapy for metastatic colorectal cancer: N16966 updated results. *Br J Cancer* 105: 58-64, 2011.
- Van Cutsem E, Rivera F, Berry S, Kretzshmar A, Michael M, DiBartolomeo M, Mazier MA, Canon JL, Georgoulis V, Peeter M, Bridgewater J, Cunningham D; First BEAT investigators: Safety and efficacy of first-line bevacizumab with FOLFOX, XELOX, FOLFIRI and fluoropyrimidines in metastatic colorectal cancer: the BEST study. *Ann Oncol* 20: 1842-1847, 2009.
- Folprecht G, Lutz MP, Schöffski P, Seufferlein T, Nolting A, Pollert P and Köhne CH: Cetuximab and irinotecan/5-fluorouracil/folinic acid is a safe combination for the first-line treatment of patients with epidermal growth factor receptor expressing metastatic colorectal carcinoma. *Ann Oncol* 17: 450-456, 2006.
- Douillard JY, Siena S, Cassidy J, Tabernero J, Burkes R, Barugel M, Humblet Y, Bodoky G, Cunningham D, Jassem J, Rivera F, Kocákova I, Ruff P, Blasińska-Morawiec M, Šmakal M, Canon JL, Rother M, Oliner KS, Wolf M and Gansert J: Randomized, phase III trial of panitumumab with infusional fluorouracil, leucovorin, and oxaliplatin (FOLFOX4) versus FOLFOX4 alone as first-line treatment in patients with previously untreated metastatic colorectal cancer: The PRIME study. *J Clin Oncol* 28: 4697-4705, 2010.
- Japanese Society for Cancer of the Colon and Rectum Japanese Classification of Colorectal Carcinoma. Second English Ed. Kanehara Co. Ltd., Tokyo, 2009.
- Allen-Mersh TG, McCullough TK, Patel H, Wharlon RQ, Glover C and Jonas SK: Role of circulating tumor cells in predicting recurrence after excision of primary colorectal carcinoma. *Br J Surg* 94: 96-105, 2007.
- Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse M, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW and Meropol NJ: Relationship of circulating tumor cells to tumor response, progression-free survival, and overall survival in patients with metastatic colorectal cancer. *J Clin Oncol* 26: 3213-3221, 2008.
- Cohen SJ, Punt CJ, Iannotti N, Saidman BH, Sabbath KD, Gabrail NY, Picus J, Morse MA, Mitchell E, Miller MC, Doyle GV, Tissing H, Terstappen LW and Meropol NJ: Prognostic significance of circulating tumor cells in patients with metastatic colorectal cancer. *Ann Oncol* 20: 1223-1229, 2009.
- Yalcin S, Kilickap S, Portakal O, Arslan C, Hascelik G and Kutluk T: Determination of circulating tumor cells for detection of colorectal cancer progression or recurrence. *Hepatogastroenterol* 57: 1395-1398, 2010.
- Kawahara H, Watanabe K, Toyama Y, Yanagisawa S, Kobayashi S and Yanaga K: Determination of circulating tumor cells for prediction of recurrent colorectal cancer progression. *Hepatogastroenterol* 59: 2115-2118, 2012.

Received February 18, 2013

Revised March 18, 2013

Accepted March 19, 2013