Review

Disseminated and Circulating Tumor Cells for Monitoring Chemotherapy in Urological Tumors

STEPHAN KRUCK*, GEORGIOS GAKIS* and ARNULF STENZL

Department of Urology, Eberhard-Karls University, Tuebingen, Germany

Abstract. This review focused on technical advancements in the detection of CTCs/DTCs in urogenital cancer. Most of the established methods of circulating tumor cell enrichment use density-gradient centrifugation and immunomagnetic procedures. Reverse transcriptase polymerase chain reaction (RT-PCR) is another detection technique. Novel methods, the CTC chip and the epithelial immunospot (EPISPOT) assay, have already shown promising results. For localized and metastatic prostate cancer, significant correlations between CTCs/DTCs and well-established indicators of disease activity have been demonstrated. Furthermore, various studies support the prognostic relevance of CTCs in metastatic bladder and renal cell carcinoma patients. Advanced technologies offer new options for estimating the risk of progression after curative-intended surgery and may allow a more effective monitoring of disease progression or response to therapy.

Despite recent advances in treatment of urogenital malignancies, including targeted therapies, the majority of patients with metastatic disease will die of their disease despite primary curative intended therapy. In many cases, effective treatment of metastatic disease is delayed due to diagnostic failure of early detection by standard clinical or radiographic evaluation (1). The presence of disseminated tumor cells at the time of primary diagnosis is assumed to be an important determinant for subsequent successful treatment and has been examined for a variety of human cancer types (2, 3). However the process of metastatic spread from the primary tumor site into distal organs is still not well understood. Recent studies suggest an early spread of tumor cells to lymph nodes or bone marrow (BM) referred to as 'disseminated tumor cells' (DTCs), or as 'circulating tumor cells' (CTCs) when present in the peripheral blood (4, 5). Even after complete removal of the primary tumor, DTCs and CTCs can cause later dissemination and development of distant metastases. There is growing evidence that CTCs indicate metastatic disease and poor prognosis (6, 7). Although the first report on CTCs was published in 1869 by Ashworth, the lack of technology precluded further investigations on their clinical use until recently (8). Today, technological advances in immunological and quantitative real-time PCR-based analysis enable clinicians to detect, enumerate and characterize DTCs and CTCs in cancer patients. The monitoring of CTCs and DTCs has the potential not only to improve therapeutic management at an early stage, but also to identify patients with increased risk of tumor progression or recurrence before the onset of clinically evident metastasis. In addition, the molecular characterization of CTCs and DTCs can provide new insights into cancer biology and systemic treatment in neo-adjuvant or adjuvant settings.

CTC and DTC detection methods. The rare presence of CTCs in the blood system (estimated as one tumor cell per billion normal blood cells) requires the use of advanced bioengineering tools for tumor cell identification and enumeration. Unfortunately, the clinical applicability of most CTC detection techniques is restricted due to their high complexity, methodological limitations and lack of standardization. DTCs and CTCs can be found in the peripheral blood (PB) and bone marrow (BM) samples of cancer patients (9). The sampling from the BM is more invasive and is difficult to implement in daily clinical practice. Current methods of CTC detection in the PB are limited because of comparatively lower CTC concentration rates (10, 11). Several methods of CTC enrichment have been investigated to increase the sensitivity (6). The most established tumor cell enrichment methods for BM and PB samples use

*Both authors contributed equally to this manuscript.

Correspondence to: Arnulf Stenzl, MD, Professor of Urology, Department for Urology, Eberhard-Karls University Tuebingen, Hoppe-Seyler-Str. 3, D – 72076 Tuebingen, Germany. Tel: +49 070712986613, Fax: +49 07071295092, e-mail: urologie@med.uni-tuebingen.de

Key Words: Circulating tumor cells, disseminated tumor cells, detection, prostate cancer, bladder cancer, renal cell carcinoma, chemotherapy, review.

density-gradient centrifugation and immunomagnetic procedures (12). Cell density-based enrichment methods are based on the principle of differential cell migration according to their buoyant density. In contrast, antibody-related techniques use specific antigen patterns on the tumor cell surface to separate tumor and blood cells. For example, tumor cell-specific cell adhesion protein EpCAM is overexpressed in many types of cancer and is absent from normal blood cells. Many approaches with EpCAM antibodies conjugated with magnetic particles followed by separation in a magnetic field have been described in literature (13, 14). After tumor cell enrichment immunological and PCR-based molecular assays are used for the detection of CTCs in order to minimize the risk of false-positive results. Some methods use only detection assays without further enrichment steps to minimize CTC loss due to limited cell separation. Immunological approaches are based on either specific epithelial (*i.e.* EpCAM) or organ-specific antigens (*i.e.* PSA) which are exclusively expressed on tumor cells and can be analyzed by simplified automated scanning devices. Automated digital ultra-speed microscopy and the use of laser scanning has opened up new opportunities for immunocytochemical approaches in this field (15).

Due to the aforementioned methods being highly labor- and time consuming, a semi-automated immunomagnetic system (CellSearch) was developed to combine both detection and enrichment of tumor cells. This technique uses ferro fluids coupled to EpCAM antibodies followed by cytokeratin staining and separation. CellSearch has already been introduced into clinical trials to monitor CTC in patients treated with new targeted therapies and was approved by the US Food and Drug Inspection Agency (16). In addition, this measuring method has been effectively used to evaluate prognostic influence on cancer progression in various types of human cancers (17-19). However EpCAM-based enrichment is limited by the wide variety of protein expression levels in different tumor types (15).

Reverse transcriptase polymerase chain reaction (RT-PCR) is one of the most commonly used techniques for CTC detection and has been successfully applied in many cancer types. This method can detect tumor-associated molecular markers with a higher sensitivity than protein-based assays (20-22). CTC detection by PCR depends on gene specific oligonucleotide primers and has already been used to detect prostate cancer cells in the PB (23). However, PCR has low specificity due to the lack of cancer-specific molecular targets in the overall majority of urogenital malignancies. In addition, tumor-associated proteins such as prostate-specific antigen (PSA) are also expressed in normal cells. Therefore, PCR is extremely susceptible to errors in quantification and needs high methodical standardization, which limits its wide clinical use.

A new detection method, the so called 'CTC-chip' can separate tumor cells from whole blood by EpCAM antibody-coated microspots. After this first step, CTCs are stained with antibodies against cytokeratin or tissue-specific markers, such as PSA in prostate cancer, and are visualized by automated scanning. The CTC chip demonstrated high sensitivity and specificity with doubled detection efficiency in comparison to currently available technologies such as CellSearch (24).

In patients with metastatic prostate cancer, the CTC chip identified 16 to 292 CTCs per milliliter. The CTC chip enables cell separation without harming the cellular integrity of tumor cells and allows subsequent molecular analysis. Another novel antibody-based technique is the epithelial immunospot (EPISPOT) assay, an adaptation of the enzyme-linked immunospot assay. This test is highly sensitive and detects only viable protein-excreting cells. In prostate cancer, the EPISPOT test detected CTCs in 65% of localized and metastasized prostate cancer patients. Patients with systemic disease showed significantly increased CTC count (25).

Prostate cancer. PSA is the most investigated biomarker across all prostate cancer disease stages. However, in many cases, PSA is inadequate to document the status of metastasis and risk of progression. The high frequency of CTCs reported in prostate cancer patients provides novel opportunities for alternative therapeutic approaches and monitoring concepts (26). In addition, recent data have shown that CTCs can be found at high frequency in metastatic disease, underlining their potential use as surrogate markers to predict clinical outcomes and survival (16, 27-33). Chen *et al.* correlated CTCs in 84 patients with advanced prostate cancer patients with PSA, prostate-specific membrane antigen expression and clinical parameters. Besides a high rate of intact CTCs, the authors found significant correlations between CTCs and established disease indicators (*i.e.* PSA, hemoglobin), but no significant correlation for Gleason score or the type of therapy and metastasis (33). In 2007, Shaffer and co-workers isolated and analyzed CTCs from PB samples of patients with advanced prostate cancer and showed that 65% of patients had five or more CTCs per 7.5 ml PB, with an average CTC count of 16. In this study CTCs were available for further analysis of epidermal growth factor receptor expression, chromosome ploidy, and androgen receptor gene amplification (32).

The role of baseline threshold CTC counts critical for survival was evaluated after immunomagnetic separation of PB from 120 patients of with progressive castration-resistant prostate cancer. Higher CTC numbers were identified predominantly in patients with bone metastasis compared to patients with soft tissue metastases and in patients who had received prior chemotherapy relative to those who had not. In univariate analysis, baseline CTCs and PSA were associated with survival without having a threshold value (28). Nagrath and co-workers established a microfluidic platform, the CTC-chip for CTC detection in

metastatic lung, prostate, pancreatic, breast and colon cancer and demonstrated a sensitivity rate of more than 99%. In early-stage prostate cancer, CTCs were isolated from all seven patients (24). In a large trial leading to the FDA approval of the CellSearch system for therapeutic monitoring of castration-resistant prostate cancer, 231 patients were stratified into two prognostic groups according to CTC count (<5 or ≥5 per 7.5 ml of PB). During a follow-up period of up to 36 months under mainly docetaxel-based chemotherapies, CTC-based prediction of overall survival was better than with PSA (16). A recent study examined potential tumor-specific aberrations in the blood of cancer patients and their use as surrogate markers for the presence of CTCs. Samples from 81 prostate cancer patients were analyzed by immunospot assay and PCR-based fluorescence microsatellite analysis using a 14 polymorphic marker panel. The authors showed correlations between the DNA plasma levels and according to tumor stage, localized and systemic disease. Moreover, CTC counts correlated with tumor stage and higher Gleason scores (34). Recently, Scher and colleagues showed a strong association between survival and CTC changes after systemic therapy in patients with metastatic, castration-resistant prostate cancer receiving first-line treatment (31). However, the low rate of CTCs in the prechemotherapy setting limits their use as a potential biomarker for prostate cancer detection. Multiple ongoing phase III studies aim to clarify the role of CTC changes and their potential to replace established biomarkers in the monitoring of chemotherapy-treated prostate cancer patients.

Renal cell carcinoma. In various studies, CTCs have also been detected in renal cell carcinoma patients in the PB by immunocytochemistry and PCR. Immunocytochemical methods have reached CTC detection rates between 32%-53% in patients with metastatic disease (9, 35-37). For PCR-based CTC analyses in renal cell carcinoma, the detection rates ranged between 37.5% and 49% (38-42). In addition, Buchner *et al*. studied 256 BM samples of nonmetastatic RCC patients and reported a detection rate of DTCs in 25% but without any prognostic relevance (43). The prognostic relevance of cytokeratin-positive BM DTCs was demonstrated in 55 patients with metastatic RCC compared with 256 patients without systemic involvement with significantly more CTCs being detected in metastatic disease (42% vs. 25%, respectively). Multivariate analysis revealed that the presence of more than 3 BM CTCs was an independent prognostic factor (44). Bluemke *et al*. evaluated the presence of CTCs in 233 PB samples from 154 patients with RCC after magnetic cell sorting followed by immunocytochemical staining against cytokeratin. In preliminary studies, the authors established a CD45 depletion protocol and identified 29%-42% of CTCs in the PB of patients with RCC. The frequency of cytokeratin-positive CTCs and 'tumor-like' blue-stained cells without cytokeratin expression showed a significant correlation to lymph node status and presence of synchronous metastases in RCC. CTCs were identified in 41% of the samples, corresponding to 53% of patients. In multivariate analysis, cytokeratin-stained CTCs were identified as an independent prognostic factor for a reduced overall survival. Interestingly, postoperative blood samples (40% of sample size) showed a higher rate of CTCs compared to preoperative ones, indicating increased tumor cell dissemination during surgery (36).

Urothelial cell carcinoma. The role of CTCs has also been studied in patients with urothelial cell carcinoma. Urothelial cancer cells express the cell surface molecule EpCAM and can therefore be detected by iron particles coated with anti-EpCAM antibodies. Naoe *et al*. demonstrated that the sensitivity of the CellSearch® Assay was approximately 79% for the detection of CTCs derived from established urothelial cancer cell lines and mixed with peripheral blood mononuclear cells. In this study, in contrast to patients with localized disease, CTCs were only present in patients with metastatic disease (45). CTCs might therefore represent an important tool to monitor the efficacy of chemotherapy in metastatic disease or addressing the risk of undiscovered micrometastatic disease when considering adjuvant chemotherapy for patients with locally advanced bladder cancer. In this respect, the feasibility of using the CellSearch® System was recently demonstrated in patients with locally advanced non-metastatic or metastatic disease. Moreover, the presence of CTCs in these patients was associated with a significantly reduced progression-free and cancer-specific survival (46). Indeed, CTCs might also help to specifically address one of the most urgent issues in bladder cancer, namely the management of patients with high-grade pT1 disease. For this stage, a defined treatment concept has not been established due to the prognostic insufficiency of clinical indicators. Recently, Gradilone *et al*. showed that the bladder cancer cell marker survivin was expressed in 92% of patient with high-grade pT1 bladder cancer and the presence of survivin-expressing CTCs was an independent predictor for decreased disease-free survival (47). It is possible that in the future, CTCs may predict the optimal timing of an aggressive surgical approach *versus* bladder-sparing treatment with bacille-Calmette-Guerin intravesical immunotherapy.

Conclusion

The systemic dissemination of tumor cells represents an important step in the development of systemic tumor spread. In urological malignancies, advanced technologies offer the opportunity to detect tumor cells in PB and BM and to estimate the risk of progression after curative intended surgery.

Despite the relatively scarce data available, DTCs and CTCs have the potential to specifically address currently controversial issues in urogenital cancer and should further be evaluated in prospective studies. In the future, CTC and DTC detection may allow a more effective monitoring of disease progression and response to therapy in daily clinical practice.

References

1 Okajima E and Kotake T: Management of advanced urogenital cancer: treatment of metastasis. Hinyokika Kiyo *40*: 905-907, 1994 (in Japanese).

2 Lugo TG, Braun S, Cote RJ, Pantel K and Rusch V: Detection and measurement of occult disease for the prognosis of solid tumors. J Clin Oncol *21*: 2609-2615, 2003.

3 Funke I and Schraut W: Meta-analyses of studies on bone marrow micrometastases: an independent prognostic impact remains to be substantiated. J Clin Oncol *16*: 557-566, 1998.

4 Pantel K and Brakenhoff RH: Dissecting the metastatic cascade. Nat Rev Cancer *4*: 448-456, 2004.

5 Husemann Y, Geigl JB, Schubert F, Musiani P, Meyer M, Burghart E, Forni G, Eils R, Fehm T, Riethmuller G and Klein CA: Systemic spread is an early step in breast cancer. Cancer Cell *13*: 58-68, 2008.

6 Paterlini-Brechot P and Benali NL: Circulating tumor cell (CTC) detection: clinical impact and future directions. Cancer Lett *253*: 180-204, 2007.

7 Pantel K, Brakenhoff RH and Brandt B: Detection, clinical relevance and specific biological properties of disseminating tumour cells. Nat Rev Cancer *8*: 329-340, 2008.

8 Asworth TR: A case of cancer in which cells similar to those in tumors were seen in the blood after death. Aust Med J *14*: 146-149, 1869.

9 Bilkenroth U, Taubert H, Riemann D, Rebmann U, Heynemann H and Meye A: Detection and enrichment of disseminated renal carcinoma cells from peripheral blood by immunomagnetic cell separation. Int J Cancer *92*: 577-582, 2001.

10 Masuda TA, Kataoka A, Ohno S, Murakami S, Mimori K, Utsunomiya T, Inoue H, Tsutsui S, Kinoshita J, Masuda N, Moriyama N and Mori M: Detection of occult cancer cells in peripheral blood and bone marrow by quantitative RT-PCR assay for cytokeratin-7 in breast cancer patients. Int J Oncol *26*: 721-730, 2005.

11 Wiedswang G, Borgen E, Schirmer C, Karesen R, Kvalheim G, Nesland JM and Naume B: Comparison of the clinical significance of occult tumor cells in blood and bone marrow in breast cancer. Int J Cancer *118*: 2013-2019, 2006.

12 Rosenberg R, Gertler R, Friederichs J, Fuehrer K, Dahm M, Phelps R, Thorban S, Nekarda H and Siewert JR: Comparison of two density gradient centrifugation systems for the enrichment of disseminated tumor cells in blood. Cytometry *49*: 150-158, 2002.

13 Trzpis M, McLaughlin PM, de Leij LM and Harmsen MC: Epithelial cell adhesion molecule: more than a carcinoma marker and adhesion molecule. Am J Pathol *171*: 386-395, 2007.

14 Wu CH, Lin SR, Hsieh JS, Chen FM, Lu CY, Yu FJ, Cheng TL, Huang TJ, Huang SY and Wang JY: Molecular detection of disseminated tumor cells in the peripheral blood of patients with gastric cancer: evaluation of their prognostic significance. Dis Markers *22*: 103-109, 2006.

15 Gerges N, Rak J and Jabado N: New technologies for the detection of circulating tumour cells. Br Med Bull *94*: 49-64, 2010.

16 de Bono JS, Scher HI, Montgomery RB, Parker C, Miller MC, Tissing H, Doyle GV, Terstappen LW, Pienta KJ and Raghavan D: Circulating tumor cells predict survival benefit from treatment in metastatic castration-resistant prostate cancer. Clin Cancer Res *14*: 6302-6309, 2008.

17 Cristofanilli M, Broglio KR, Guarneri V, Jackson S, Fritsche HA, Islam R, Dawood S, Reuben JM, Kau SW, Lara JM, Krishnamurthy S, Ueno NT, Hortobagyi GN and Valero V: Circulating tumor cells in metastatic breast cancer: biologic staging beyond tumor burden. Clin Breast Cancer *7*: 471-479, 2007.

18 Naoe M, Ogawa Y, Takeshita K, Iwamoto S and Miyazaki A: Use of the Cell Search Circulating Tumor Cell Test for monitoring urothelial cancer: two case reports of metastatic urothelial cancer. South Med J *101*: 439-441, 2008.

19 Wind J, Tuynman JB, Tibbe AG, Swennenhuis JF, Richel DJ, van Berge Henegouwen MI and Bemelman WA: Circulating tumour cells during laparoscopic and open surgery for primary colonic cancer in portal and peripheral blood. Eur J Surg Oncol *35*: 942-950, 2009.

20 Ghossein RA, Scher HI, Gerald WL, Kelly WK, Curley T, Amsterdam A, Zhang ZF and Rosai J: Detection of circulating tumor cells in patients with localized and metastatic prostatic carcinoma: clinical implications. J Clin Oncol *13*: 1195-1200, 1995.

21 Seiden MV, Kantoff PW, Krithivas K, Propert K, Bryant M, Haltom E, Gaynes L, Kaplan I, Bubley G, DeWolf W *et al*: Detection of circulating tumor cells in men with localized prostate cancer. J Clin Oncol *12*: 2634-2639, 1994.

22 Bossolasco P, Ricci C, Farina G, Soligo D, Pedretti D, Scanni A and Deliliers GL: Detection of micrometastatic cells in breast cancer by RT-pCR for the mammaglobin gene. Cancer Detect Prev *26*: 60-63, 2002.

23 Helo P, Cronin AM, Danila DC, Wenske S, Gonzalez-Espinoza R, Anand A, Koscuiszka M, Vaananen RM, Pettersson K, Chun FK, Steuber T, Huland H, Guillonneau BD, Eastham JA, Scardino PT, Fleisher M, Scher HI and Lilja H: Circulating prostate tumor cells detected by reverse transcription-PCR in men with localized or castration-refractory prostate cancer: concordance with CellSearch assay and association with bone metastases and with survival. Clin Chem *55*: 765-773, 2009.

24 Nagrath S, Sequist LV, Maheswaran S, Bell DW, Irimia D, Ulkus L, Smith MR, Kwak EL, Digumarthy S, Muzikansky A, Ryan P, Balis UJ, Tompkins RG, Haber DA and Toner M: Isolation of rare circulating tumour cells in cancer patients by microchip technology. Nature *450*: 1235-1239, 2007.

25 Alix-Panabieres C, Vendrell JP, Pelle O, Rebillard X, Riethdorf S, Muller V, Fabbro M and Pantel K: Detection and characterization of putative metastatic precursor cells in cancer patients. Clin Chem *53*: 537-539, 2007.

26 Allard WJ, Matera J, Miller MC, Repollet M, Connelly MC, Rao C, Tibbe AG, Uhr JW and Terstappen LW: Tumor cells circulate in the peripheral blood of all major carcinomas but not in healthy subjects or patients with nonmalignant diseases. Clin Cancer Res *10*: 6897-6904, 2004.

27 Moreno JG, Miller MC, Gross S, Allard WJ, Gomella LG and Terstappen LW: Circulating tumor cells predict survival in patients with metastatic prostate cancer. Urology *65*: 713-718, 2005.

28 Danila DC, Heller G, Gignac GA, Gonzalez-Espinoza R, Anand A, Tanaka E, Lilja H, Schwartz L, Larson S, Fleisher M and Scher HI: Circulating tumor cell number and prognosis in progressive castration-resistant prostate cancer. Clin Cancer Res *13*: 7053-7058, 2007.
29 Halabi S, Vogelzang NJ, Kornblith AB, Ou SS, Kantoff PW, Dawson NA and Small EJ: Pain predicts overall survival in men with metastatic castration-refractory prostate cancer. J Clin Oncol *26*: 2544-2549, 2008.
30 Ramiah V, George DJ and Armstrong AJ: Clinical endpoints for drug development in prostate cancer. Curr Opin Urol *18*: 303-308, 2008.
31 Scher HI, Jia X, de Bono JS, Fleisher M, Pienta KJ, Raghavan D and Heller G: Circulating tumour cells as prognostic markers in progressive, castration-resistant prostate cancer: a reanalysis of IMMC38 trial data. Lancet Oncol *10*: 233-239, 2009.
32 Shaffer DR, Leversha MA, Danila DC, Lin O, Gonzalez-Espinoza R, Gu B, Anand A, Smith K, Maslak P, Doyle GV, Terstappen LW, Lilja H, Heller G, Fleisher M and Scher HI: Circulating tumor cell analysis in patients with progressive castration-resistant prostate cancer. Clin Cancer Res *13*: 2023-2029, 2007.
33 Chen BT, Loberg RD, Neeley CK, O'Hara SM, Gross S, Doyle G, Dunn RL, Kalikin LM and Pienta KJ: Preliminary study of immunomagnetic quantification of circulating tumor cells in patients with advanced disease. Urology *65*: 616-621, 2005.
34 Schwarzenbach H, Alix-Panabieres C, Muller I, Letang N, Vendrell JP, Rebillard X and Pantel K: Cell-free tumor DNA in blood plasma as a marker for circulating tumor cells in prostate cancer. Clin Cancer Res *15*: 1032-1038, 2009.
35 Meye A, Bilkenroth U, Schmidt U, Fussel S, Robel K, Melchior AM, Blumke K, Pinkert D, Bartel F, Linne C, Taubert H and Wirth MP: Isolation and enrichment of urologic tumor cells in blood samples by a semi-automated CD45 depletion autoMACS protocol. Int J Oncol *21*: 521-530, 2002.
36 Bluemke K, Bilkenroth U, Meye A, Fuessel S, Lautenschlaeger C, Goebel S, Melchior A, Heynemann H, Fornara P and Taubert H: Detection of circulating tumor cells in peripheral blood of patients with renal cell carcinoma correlates with prognosis. Cancer Epidemiol Biomarkers Prev *18*: 2190-2194, 2009.
37 Blumke K, Bilkenroth U, Schmidt U, Melchior A, Fussel S, Bartel F, Heynemann H, Fornara P, Taubert H, Wirth MP and Meye A: Detection of circulating tumor cells from renal carcinoma patients: experiences of a two-center study. Oncol Rep *14*: 895-899, 2005.
38 Hioki T and Sugimura Y: Detection of circulating cancer cells by nested reverse transcription-polymerase chain reaction of cytokeratin-19 in patients with renal cell carcinoma. Hinyokika Kiyo *45*: 577-581, 1999 (in Japanese).
39 McKiernan JM, Buttyan R, Bander NH, de la Taille A, Stifelman MD, Emanuel ER, Bagiella E, Rubin MA, Katz AE, Olsson CA and Sawczuk IS: The detection of renal carcinoma cells in the peripheral blood with an enhanced reverse transcriptase-polymerase chain reaction assay for MN/CA9. Cancer *86*: 492-497, 1999.
40 Shimazui T, Yoshikawa K, Uemura H, Kawamoto R, Kawai K, Uchida K, Hirao Y, Saga S and Akaza H: Detection of cadherin-6 mRNA by nested RT-PCR as a potential marker for circulating cancer cells in renal cell carcinoma. Int J Oncol *23*: 1049-1054, 2003.
41 Ashida S, Okuda H, Chikazawa M, Tanimura M, Sugita O, Yamamoto Y, Nakamura S, Moriyama M and Shuin T: Detection of circulating cancer cells with von Hippel-Lindau gene mutation in peripheral blood of patients with renal cell carcinoma. Clin Cancer Res *6*: 3817-3822, 2000.
42 Li G, Passebosc-Faure K, Gentil-Perret A, Lambert C, Genin C and Tostain J: Cadherin-6 gene expression in conventional renal cell carcinoma: a useful marker to detect circulating tumor cells. Anticancer Res *25*: 377-381, 2005.
43 Buchner A, Riesenberg R, Kotter I, Crispin A, Hofstetter A and Oberneder R: Detection and prognostic value of cytokeratin positive tumor cells in bone marrow of patients with renal cell carcinoma. J Urol *170*: 1747-1751, 2003.
44 Buchner A, Riesenberg R, Kotter I, Hofstetter A, Stief C and Oberneder R: Frequency and prognostic relevance of disseminated tumor cells in bone marrow of patients with metastatic renal cell carcinoma. Cancer *106*: 1514-1520, 2006.
45 Naoe M, Ogawa Y, Morita J, Omori K, Takeshita K, Shichijyo T, Okumura T, Igarashi A, Yanaihara A, Iwamoto S, Fukagai T, Miyazaki A and Yoshida H: Detection of circulating urothelial cancer cells in the blood using the CellSearch System. Cancer *109*: 1439-1445, 2007.
46 Rink M, Chun FK, Minner S, Friedrich M, Mauermann O, Heinzer H, Huland H, Fisch M, Pantel K and Riethdorf S: Detection of circulating tumour cells in peripheral blood of patients with advanced non-metastatic bladder cancer. BJU Int *107*: 1668-1675, 2011.
47 Gradilone A, Petracca A, Nicolazzo C, Gianni W, Cortesi E, Naso G, Vincenzi B, Cristini C, De Berardinis E, Di Silverio F, Agliano AM and Gazzaniga P: Prognostic significance of survivin-expressing circulating tumour cells in T1G3 bladder cancer. BJU Int *106*: 710-715, 2010.

Received March 18, 2011
Revised May 15, 2011
Accepted May 17, 2011