Utility of Measuring Circulating Tumor Cell Counts to Assess the Efficacy of Treatment for Carcinomas of Unknown Primary Origin

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Abstract. Background/Aim: Carcinomas of unknown primary origin (CUPs) account for 3%-5% of all malignancies. The majority of CUPs have unfavorable prognosis and are chemoresistant. Predictive biomarkers should be established to improve therapeutic outcomes. Metastatic ability of CUPs may be related to the existence of circulating tumor cells (CTCs). Patients and Methods: Ten patients diagnosed with CUP visiting the Akita University Hospital participated in this study. CTCs were calculated by the CellSearch system. Results: The present observational study indicates that CTCs were detected in 50% of CUPs, and in 80% chemotherapeutically-naïve cases. Furthermore, decrease in CTC count between the pre-treatment and post-treatment phases were observed in chemosensitive cases. Conclusion: Rapid assessment of the efficacy of chemotherapy by CTC count may become a useful predictive biomarker of CUPs.

Carcinomas of unknown primary origin (CUP) are defined as histopathologically-confirmed metastatic carcinomas with no primary site identified after completion of clinical examinations (1). Favorable subsets of CUP, including extra-gonadal germ cell cancer, peritoneal papillary adenocarcinoma, adenocarcinoma of the axillary lymph nodes (LN), cervical squamous cell carcinoma, neuroendocrine carcinoma (NEC), and bone metastasis with elevated prostate-specific antigen levels, are sensitive to the same chemotherapies as the supposed corresponding primaries (1). However, these subsets account for only 15% of all CUPs. The remaining 85% are considered to comprise an unfavorable subset, for which the reported response rate is 11.7%-65.1% and the median survival time is 6-10 months (1). Difficulty in predicting the primary site for unfavorable subsets is likely responsible for poorer therapeutic outcomes. Thus, the development of comprehensive predictive biomarkers for all relevant therapeutic agents is very important.

Recent studies have reported the successful isolation of circulating tumor cells (CTC) in metastatic malignancies (2-4). Because metastases are characteristic of CUP, patients with CUP are considered to have high CTC counts. The CellSearch system (Veridex LLC, Raritan, NJ, USA) was approved for determination of CTC count by the US Food and Drug Administration. CTC analyses have been included in several clinical trials, yielding promising results (5, 6). In this study, we hypothesized that changes in CTC counts between pre-treatment and post-treatment phases with patients with CUP could be a predictive biomarker for the efficacy of ongoing treatment. To our knowledge, the literature contains no publications describing CTC count variations in CUP.

Patients and Methods

Ten patients diagnosed with CUP and treated at the Department of Clinical Oncology, Akita University Hospital from October 2012 to October 2013 were enrolled. This study was ethically-approved by the Committee of the School of Medicine of the Akita University (#1055). Written informed consent and agreement to publication were obtained from all participants of this cohort study.

Isolation of CTCs. The method used to isolate CTCs is fully-described elsewhere (8). In brief, CTCs were isolated from 20 ml of peripheral venous blood drawn from each patient, using the CellSearch kit and the CellTracks AutoPrep system (Veridex LLC). CTCs were characterized using the CellTracks Analyzer (Veridex LLC).

Key Words: Biomarker, carcinomas of unknown primary origin, chemotherapy, circulating tumor cell, prediction.
Demographic information on the CUP cohort is presented in Table 1. Patients included 6 males with an age range of 47-83 years (median=65 years). CUP histology was as follows: adenocarcinoma (n=7), spindle cell carcinoma (n=2), and NEC (n=1). Five patients were chemotherapeutically-naïve. The overall CTC detection rate was 50.0%. It was particularly high (80%, 4/5) in chemotherapeutically-naïve cases. The range of the CTCs counts per 7.5 ml of whole blood was 3-207 (median=31) at initial examination.

Among five CTC-positive patients, one did not receive chemotherapy because of his age (83 years), whereas another agreed to undergo CTC examination only once. Case 1 was a 59-year-old female (Figure 1). In September 2012, cervical LN swelling was observed and was confirmed as adenocarcinoma, but the tumor was estrogen and progesterone receptor-negative. She was referred to our Department on October 29, 2012, when 18F-fluorodeoxyglucose positron-emission tomography–computed tomography (PET-CT) revealed metastases to all lumbar vertebrae, sacrum, left subclavian LN, and right axillary LN [standardized uptake value (SUV)_max 4.0-5.0]. The CTC count was 207/7.5 ml whole blood. On November 16, 2102, carcinoembryonic antigen (CEA) was elevated to 77.1 ng/ml, carbohydrate antigen (CA)19-9 to 835.3 U/ml (normal range <37 U/ml), and CTC to 995/7.5 ml whole blood. Chemotherapy with epirubicin and cyclophosphamide (EC) was initiated. Two weeks later, CEA decreased to zero, but progastrin-releasing peptide (Pro-GRP) remained high at 1929 pg/ml (normal range, <81). CT performed on August 14, 2013 confirmed the status as being close to complete response (CR) by the Response Evaluation Criteria in Solid Tumors, version 1.1. for most target lesions except the left adrenal gland (74% reduction) and brain metastases, and Pro-GRP had normalized to 49 pg/ml. CE therapy was continued monthly for six cycles. Surveillance by CT and CTC conducted on October 30, 2013 confirmed the same status, and Pro-GRP was normal (57.4 pg/ml). In this case, the CTC count indicated the positive effect of CE treatment nine weeks earlier than by imaging.

Case 2 was a 66-year-old male (Figure 2). He began to lose spatial perception in March 2013. Brain magnetic resonance imaging revealed a tumor in the right parietal lobe, and more than 20 nodular tumors (all <20 mm in diameter) were found disseminated in the cerebrum and cerebellum. CT indicated the swelling of two peripancreatic LN, a tumor in each adrenal gland, and a tumor in the left cervical region. All were considered metastatic lesions, but no primary was detected. He was referred to our Department on May 28, 2013. Abdominal LN histopathology showed small cell-like appearance. Cells were chromogranin A-, CD56- and synaptophysin-positive, and 80% of them were Ki67-positive. These findings led to the diagnosis of NEC. Whole-brain radiotherapy (30 Gy/10 fractions) was started on April 13, 2013, and chemotherapy with carboplatin and etoposide (CE) was started on May 20, 2013, when CTC counts were 31/7.5 ml whole blood. Three weeks later, CTC counts decreased to zero, but progastrin-releasing peptide (Pro-GRP) remained high at 1929 pg/ml (normal range, <81). CT performed on August 14, 2013 confirmed the status as being close to complete response (CR) by the Response Evaluation Criteria in Solid Tumors, version 1.1. for most target lesions except the left adrenal gland (74% reduction) and brain metastases, and Pro-GRP had normalized to 49 pg/ml. CE therapy was continued monthly for six cycles. Surveillance by CT and CTC conducted on October 30, 2013 confirmed the same status, and Pro-GRP was normal (57.4 pg/ml). In this case, the CTC count indicated the positive effect of CE treatment nine weeks earlier than by imaging.

Case 3 was a 64-year-old female. She had multiple bone metastases involving the ribs, the vertebrae, and the right

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (years)/gender</th>
<th>Histology</th>
<th>Metastasis</th>
<th>CTC (/7.5 ml)</th>
<th>Previous treatment</th>
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<td>Bone, LN</td>
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<td>Naïve</td>
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<tr>
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<td>Plc, LN</td>
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<td>CBDCA+DTX</td>
</tr>
<tr>
<td>5</td>
<td>66/M</td>
<td>NEC</td>
<td>Bone, brain, PC</td>
<td>31</td>
<td>Naïve</td>
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<td>Bone, LN</td>
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</tr>
</tbody>
</table>

Figure 1. Clinical course of case 1. A: Changes in circulating tumor cell (CTC) count (yellow bars), and carbohydrate antigen 19-9 (CA19-9) plotted at three time points. B and C: Images showing bone metastases on the dates indicated.

Figure 2. Clinical course of case 2. A: Changes in circulating tumor cell (CTC) count (yellow bars) and progastrin-releasing peptide (Pro-GRP) plotted at three time points. B and C: Images showing abdominal tumors (*) on the dates indicated.
Publications

References


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Discussion

The present study results show that CTCs can be detected in patients with CUP at a rate considerably higher than that in our previous analysis on metastatic colorectal carcinoma (38.5%) (7). CTC counts in CUP were also greater than those observed in metastatic colorectal carcinoma (median=1) (7).

In the selection of appropriate chemotherapy, two major approaches are now adopted: (i) prediction of the latent primary CUP site by expression analysis using methods such as CupPrint®; (ii) identification of target molecules in CUP (8, 9).

CTCs are considered to comprise several entities, and this heterogeneity might create a problem in the analysis of archival specimens (1). Real-time sampling, (e.g. CTC counts or circulating tumor DNA) was considered to be important (10). In the present study, changes in CTC count were apparent within a few weeks of chemotherapy initiation. Confirmation by RECIST, on the other hand, takes at least two months, and if the treatment proves ineffective, this may be unnecessarily prolonged. Although further study is required because of our limited sample size, CTC analysis seems useful in CUP.

Conflicts of Interest

There are no conflicts of interest to declare.

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