The Surge in the Number of Circulating Tumor Cells Following Treatment with Sunitinib for Metastatic Renal Cell Carcinoma

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Abstract:
Circulating tumor cells (CTCs) are a promising biomarker for several cancers. We streamlined the experimental procedure of CTC immunofluorescent staining. We encountered a 72-year-old woman with metastatic right renal cell carcinoma (RCC) (clinical stage: T4N0M1), whose CTC number rapidly increased after the administration of sunitinib and then gradually decreased. The change in the CTC number appeared to coincide with laboratory data and hypertension, suggesting that a CTC analysis may be useful for promptly monitoring the treatment response. Our data provided the first evidence of an association between the CTC numbers and the treatment response in a metastatic RCC patient.

Key words: circulating tumor cells, renal cell carcinoma, sunitinib

Introduction

Radiological examinations and serum tumor markers are mainstays for disease monitoring. Therefore, the absence of specific tumor markers for renal cell carcinoma (RCC) together with the limitations of imaging scans make real-time monitoring of RCC progression challenging, giving rise to the need for a new detection method in clinical practice.

Recently, several molecular targeting agents, such as tyrosine kinase inhibitors (TKIs) and mammalian target of rapamycin inhibitors, have become widely available for metastatic RCC. However, the selection of an appropriate drug depends on the clinician’s experience rather than biological evidence.

Several reports have shown that circulating tumor cells (CTCs) are a promising biomarker for several cancers (1-4), as CTCs appear to represent cancer characteristics and are associated with the clinical course. In addition, serial CTC monitoring is expected to be a predictive biomarker of treatment response in other cancer types (5-7).

At our institution, we streamlined the experimental procedure of CTC immunofluorescent staining so that it can be completed within a day. In the present study, we evaluated whether or not a CTC analysis is useful as a new clinical biomarker in progressive metastatic RCC.

Case Report

A 72-year-old woman was referred to our hospital because of weight loss and jaundice on 25 April 2016. Because of jaundice, she presented with yellowish discoloration of the bulbar conjunctiva and the skin. Computed tomography (CT) revealed right RCC. The size of the tumor was 30.3 cm² in area measured, as by the Image-J software program, and directly invaded the inferior vena cava (IVC), the liver, and the right adrenal gland and metastasized to the lung (Fig. 1A and D). The clinical stage was T4N0M1. Notably, a radiological report indicated that the tumor did not show typical characteristics of clear cell RCC, such as early strong enhancement and a washout pattern. Instead, other histological types, particularly papillary type 2 RCC, were more likely because of the robust local invasion.

At the initial visit, the serum total bilirubin (T-Bil: 4.29 mg/dL) and serum lactate dehydrogenase (LDH: 161 U/L) exceeded the upper normal limits. Among the adverse prog-
Figure 1. Computed tomography (CT) images during the clinical course. (A) Contrast-enhanced abdominal CT image before the treatment. The area of the tumor was 30.3 cm², measured at the level of the branching of the right renal artery. The analysis was done using the Image-J software program. This image shows that tumor directly invaded the right renal vein and the inferior vena cava (IVC). Scale bars, 2 cm. (B) Contrast-enhanced abdominal CT image on Day 20 after the treatment. The area of the tumor was 26.1 cm², measured at the level of the branching of the right renal artery. Scale bars, 2 cm. (C) Plain abdominal CT image on Day 128 after treatment. The area of the tumor was 17.6 cm², measured at the level of the branching of the right renal artery. The analysis was done using the Image-J software program. This image shows a 42% reduction in tumor size compared with pretreatment. Scale bars, 2 cm. (D) Chest CT image of lung metastasis. The arrowheads indicate the lung metastasis. The left image shows pre-treatment left lung metastasis (9.8×8.8 mm). The right image shows the same metastasis on Day 128 after the treatment with sunitinib. Scale bars, 2 cm.
The detection of CTC was done using a CytoQuest CR (Ab-
nova, Taipei, Taiwan) (Fig. 2) in accordance with the manu-
facturer’s protocol. The Cytoquest CR positively selects
CTCs using anti-epithelial-cell-adhesion molecule (Ep-
CAM)-capturing antibody. First, 4.0 mL of blood was drawn
into an EDTA-2Na collection tube. Next, buffy coat contain-
ning CTCs was separated by gradient cell separation. After
washing with RPMI medium, the prepared buffy coat was
injected into an EpCAM-coated microfluidic slide. The cap-
tured cells were immunostained with the antibody cocktail
containing anti-pan-cytokeratin (PanCK) antibody (FITC),
anti-CD45 antibody (PE), and DAPI. Stained cells were ob-
served with a fluorescence microscope (Keyence, Osaka, Ja-
pan). The CTCs were defined as PanCK (+), CD45 (-), and
DAPI (+) cells, whereas white blood cells (WBCs) were de-
cscribed with a fluorescence microscope (Keyence, Osaka, Ja-
pan). The CTC count was done manually before sunitinib admin-
istration and on Days 2, 4, 10, and 20 during treatment to
monitor the change in CTC number.

We detected CTCs before sunitinib treatment and on Days
2, 4, 10, and 20 during the treatment. Details on the CTC
number, blood pressure, and laboratory data such as LDH,
monocyte and T-Bil are shown in (Fig. 4). Following the ad-
ministration of sunitinib, the CTC number increased rapidly
on Day 2, presumably due to an oncolytic event, and then
decreased gradually from Day 4 until Day 20, followed by a
slight and transient increase on Day 10. Regarding the labo-
atory data, immediately after the administration of sunit-
inib, there were transient increases in the LDH and mono-
cyte values. T-Bil decreased soon after the treatment but in-
creased transiently on Day 10, similar to the CTC number
and other laboratory data. On Day 20, the CTC number and
LDH dropped below the pre-treatment levels, and T-Bil de-
clined to 2.5 mg/dL These improvements in data allegedly
occurred as a result of the relief of tumor occlusion in the
IVC.

In terms of adverse events, grade 3 asymptomatic hyper-
tension according to the Criteria for Adverse Events (NCI
CTCAE, version 4.0) occurred on Days 2 and 10, and grade

![Figure 2](image_url) **Figure 2.** The semi-automatic CytoQuest CR to enrich CTC. The CytoQuest CR captures CTC from buffy coat in EpCAM-coated slide. CTC: circulating tumor cell, EpCAM: epithelial-cell-adhesion molecule

![Figure 3](image_url) **Figure 3.** Immunofluorescent staining of CTCs and WBCs. Immunofluorescent images of CTCs and WBCs collected by CytoQuest CR are shown. The CTCs are captured on an EpCAM-coated slide. The CTCs stain positive for PanCK, an epithelial maker, and negative for CD45, a hematopoietic marker. The WBCs stain negative for PanCK and positive for CD45. CTCs: circulating tumor cells, EpCAM: epithelial-cell-adhesion molecule, PanCK: pan-cytokeratin, WBC: white blood cells
Figure 4. Clinical course, changes in the laboratory data and CTC number. The CTC analysis and routine blood tests were conducted before the treatment and on Days 2, 4, 10, and 20 during the treatment. Down-pointing arrows indicate CTC analysis points. Sunitinib administration is indicated with blue bars (37.5 mg/day for 2 weeks followed by 1 week off). The vertical axis on the left shows the values of monocytes, lactate dehydrogenase (LDH), and systolic blood pressure. The vertical axis on the right shows the values of total bilirubin (T-Bil). The CTC numbers are indicated with red bars with the actual numbers on the top. (B) Low-magnitude images of CTCs before sunitinib administration and at Days 2, 4, 10, and 20 after treatment. The white arrows indicate CTCs, and aggregated CTCs are circled. Only merged images are shown in this figure. CTCs: circulating tumor cells, LDH: lactate dehydrogenase
2 hypothyroidism occurred on Day 87 (TSH 35.9 μIU/mL, FT3 2.5 pg/mL, FT4 1.0 ng/dL). An antihypertensive drug (amlodin 5 mg) was prescribed on Day 2, and the blood pressure returned to normal after Day 10. Thus, sunitinib was continued without dose reduction.

A CT examination on Day 128 showed a 14% reduction in the size of the primary lesion with shrinkage of the lung metastasis (Fig. 1D). In addition, the primary lesion further shrunk by 28% (Fig. 1A-C). The area of the tumor was measured at the level of branching of the right renal artery using the Image-J software program.

**Discussion**

In the present patient, we performed a CTC analysis along with a routine blood examination and CT for real-time monitoring of the disease status. In order to capture rare CTCs in the peripheral blood, we employed a CTC detection device, the CytoQuest CR, which enabled the detection of CTCs within a day after blood collection. The CTC number rapidly increased after sunitinib administration and gradually decreased afterwards. Given the concurrent adverse events and concordant fluctuations in some laboratory data, the changes in the CTC number may reflect the treatment efficacy.

T-Bil served as a biomarker of the treatment response in the present patient, as the direct tumor invasion to the liver resulted in a deteriorated liver function. The concordant fluctuation of the CTC number with the T-Bil value indicated that the monitoring of the CTC number is a promising biomarker for predicting the treatment response.

Regarding adverse events, a rise in blood pressure (Day 2) appeared to coincide with an increase in the CTC number, in addition to hypothyroidism that occurred in the late course of treatment. Since the occurrence of adverse events is reported to be associated with an improved clinical outcome in patients receiving sunitinib (11), we surmise that sunitinib exerted anti-cancer effects on the tumor, including the one projecting into the IVC, leading to the influx of dissociated cancer cells into the circulation. Together, the adverse events and increase in CTC number may be indicative of a favorable outcome. In line with our hypothesis, the tumor size decreased in a time-dependent manner (Fig. 1B and C). Consistent with our findings, several earlier studies reported that CTCs more promptly reflect the treatment response than current imaging techniques (12).

However, CTC detection is generally difficult in RCC patients due to the low expression of EpCAM, especially in clear cell RCC, which accounts for the majority of RCC cases (13). In our study, despite the fact that EpCAM was targeted to capture RCC-derived CTCs, we were still able to capture a number of CTCs, possibly due to the pathological features of this case of RCC, as our patient did not present with the typical characteristics of clear cell RCC on CT. Indeed, an earlier study reported that chromophobe and papillary type RCC express more EpCAM than clear cell type (13).

Regarding the utility of CTC as a novel biomarker, Blumke et al. reported that the detection of cytokeratin-positive CTC in metastatic RCC patients was a poor independent prognostic factor (14). Furthermore, in other types of cancers, such as prostate cancer and colorectal cancer, intensive studies have shown that CTC enumeration using the CellSearch system is reflective of the treatment response and prognosis (15, 16). However, the real-time monitoring of the CTC number following treatment was not performed in earlier studies. Although we agree that the presence of CTC per se reflects disease aggressiveness, our results suggest that an increase in the CTC number as a result of an oncolytic event may be a favorable biomarker. In addition, single cell pick-up from the slide followed by whole-genome amplification allows for the detection of loss-of-function mutations in key genes, such as CDKN2A and fumarate hydratase, in our assay (17).

Several limitations associated with this study must be considered. First, there may be some observer bias when counting the CTC number. To eliminate this bias, observers were not notified of the treatment course. Second, the positive selection of CTCs requires EpCAM expression on the surface of CTCs, which, in turn, eliminates EpCAM-negative CTCs that underwent epithelial-mesenchymal transition. Furthermore, due to the lack of a consensus on RCC markers, a molecular analysis of CTC could not be performed in this study. Finally, a pathological diagnosis was not made in this case, since surgical resection was not performed due to an inoperable tumor burden.

Our data provided the first evidence of an association between the number of CTCs and the treatment response in a metastatic RCC patient. A large-scale study is warranted for the validation of these findings.

**Author’s disclosure of potential Conflicts of Interest (COI).**

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**References**

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