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Research Article

Arterial Blood, Rather Than Venous Blood, is a Better Source for Circulating Melanoma Cells

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ABSTRACT

Background: CTCs provide prognostic information and their application is under investigation in multiple tumor types. Of the multiple variables inherent in any such process, none is more important to outcome than the appropriateness of the sample source. To address this question, we investigated CTCs in paired peripheral venous and arterial blood specimens obtained from stage IV uveal melanoma patients.
Methods: Blood specimens were obtained from both common femoral arteries and antecubital veins in 17 uveal melanoma patients with multiple hepatic metastases for CTC measurements.
Finding: CTCs were detectable with greater frequency (100%) and in larger numbers (median 5, range 1 to 168) in all arterial blood specimens than in venous samples (52.9%; median 1, range 0 to 8). Patients with hepatic as well as extra-hepatic metastasis showed higher number of arterial CTCs, compared to patients with liver-only metastasis ($p = 0.003$). There was no significant association between the number of arterial CTCs and the tumor burden within the liver in patients who had liver-only metastases.
Interpretation: Our data indicate that arterial blood specimens might be a better source of circulating uveal melanoma cells. Although less conveniently processed, perhaps arterial blood should be evaluated as sample source for measurement of CTCs.

1. Introduction

Uveal melanoma (UM) is the most common primary cancer of the eye in adults, with a reported incidence of 5.1 per million (Singh et al., 2011). The majority of UM cases (97.8%) occur in the Caucasian population (Singh et al., 2011). Despite the common embryologic origin of cutaneous and uveal melanocytes, the clinical, epidemiologic, and molecular characteristics of UM differ from those of cutaneous melanoma (Collaborative Ocular Melanoma Study G, 2001; Singh et al., 2001; Ewens et al., 2014). Local treatment of primary UM has improved; conservative non-surgical treatments such as brachytherapy with radioisotopes result in eye preservation and control the growth of primary UM. However, this improvement in local treatment did not significantly increase the overall survival for UM patients (Singh et al., 2011). Systemic metastases develop in up to 50% of the cases of UM patients. UM disseminates hematogeneously, as there is no major lymphatic drainage from the eye. Metastatic disease leads to death in the majority of patients because of the lack of effective systemic treatments (Kujala et al., 2003). The metastatic UM cells have significant tropism to the liver, and the liver is the first organ of metastasis in approximately 80% to 95% of patients who develop systemic recurrence. Several histologic, genetic, and demographic factors have been associated with metastases in UM, including large tumor size in primary cancer of the eye, monosomy 3, and BAP1 mutation (Collaborative Ocular Melanoma Study G, 2001; Ewens et al., 2014). It has been reported that 80% of metastatic uveal melanoma have mutation in BAP1 (Harbour et al., 2010). Published clinical observations suggested that UM cell metastases in the liver grow faster than metastases in other organs (All-Ericsson et al., 2002; Yoshida et al., 2014; Chattopadhyay et al., 2014). The lung is the second most common site of metastasis. A small percentage of patients first develop osseous and brain metastasis (Lorigan et al., 1991; Rietschel et al., 2005). It has been reported that distant micrometastasis resulting from the dissemination of tumor cells

Abbreviations: Ab, antibody; AKTi, AKT inhibitor; BCNU, bischlorethylnitrosourea; DEBDOX, drug-eluting beads with doxorubicin; EDTA, ethylenediaminetetraacetic acid; HMW-MAA, high molecular weight melanoma associated antigen; Ipi, ipilimumab; LN, lymph node; MEKi, MEK inhibitor; METi, MET inhibitor;; TACE, transarterial chemoembolization; VPA, valproic acid; XRT, radiation therapy.

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through the blood stream developed even before primary UM was clinically diagnosed and treated (Eskelin et al., 2000). It is also reported that the recurrence for patients undergoing enucleation displays a bimodal pattern, peaking three years with a second surge peaking at about nine years (Demicheli et al., 2014).

Due to their buoyancy, circulating tumor cells (CTCs) are found in the white blood cell fraction. CTCs have been investigated as a non-radiographic tool to monitor disease progression. The presence of CTCs suggests increased metastatic potential, and they have been investigated as a predictive marker for systemic recurrence. They could also serve as a source for diagnostic testing (liquid biopsy) in cases where the biopsy of metastases is difficult or risky. In such cases, evaluation of CTCs in blood would be more convenient and could be useful in obtaining critical information on the biological characteristics of cancer cells to facilitate a diagnostic or therapeutic decision. Furthermore, the genomic profile of CTCs may predict homing and colonization to specific distant organ sites (Li et al., 2008; Burger and Kipps, 2006).

CTCs have been detected in the majority of epithelial cancers, including those from the prostate (Danila et al., 2007), colon/rectum (Cohen et al., 2008), and breast (Cristofanilli et al., 2004). In patients with metastatic breast cancer, CTC counts above 5 per 7.5 ml of venous blood before the start of systemic therapy are associated with shorter median progression-free and overall survivals (Cristofanilli et al., 2008). The key mutation for therapeutic resistance has been found in CTCs in metastatic breast cancer patients and CTCs could potentially be used as a predictable marker for treatment response and resistance (Fernandez et al., 2014). In fact, the CellSearch® System was approved by the United States (US) Food and Drug Administration (FDA) for monitoring treatment effectiveness in metastatic prostate, colorectal, and breast cancer patients.

Venous blood collection is simple and minimally invasive, and this approach has made CTC testing readily available to many cancer patients. The major drawback is the fact that CTCs are not always detectable for patients with clinically evident metastatic disease. This observation raises the concern that a number of CTCs might have been sequestered or destroyed while circulating in the blood stream. Alternatively, it is possible that CTCs may have been lost during analysis due to technical reasons.

Recently, the field of CTC detection technologies has been significantly improved and various new approaches have been developed including filtration (Mazzini et al., 2014), dual immunomagnetic enrichment assay (Tura et al., 2014), fiber-optic array scanning (Krivacic et al., 2004), microfluidics (Dong et al., 2013) and photoacoustic-flow cytometry (Menyaev et al., 2013; Sarimollaoglu et al., 2014). However, none of these new technologies have been validated for approval by the US FDA.

Although accumulative evidence suggests that CTCs could provide prognostic information in breast cancer patients, the clinical benefits in measuring CTCs in UM patients remain controversial. It has been shown that the detection of CTCs in venous blood specimens of primary UM patients prior to their local treatment was 14% with an immunomagnetic enrichment method. However, there was no significant difference between the number of CTCs before and after their local therapies, and the number of CTCs was not correlated to the development of metastasis in a short median follow-up time of 16 months (Suesskind et al., 2011). Bidard et al. reported the result of CTC detection with the CellSearch® method in 40 stage IV UM patients with liver metastasis, in which eight out of 40 patients exhibited additional extra-hepatic metastasis. Surprisingly, no CTCs were detected in 70% of patients with hepatic metastasis. The median number of CTCs was 3 and the range of CTCs was 1 to 20 in 12 patients who showed positive CTCs in their venous blood specimens (Bidard et al., 2014). These results indicate that CTC measurement in venous blood may not be useful in stage IV UM patients since the detection rate of CTCs is very low. This also raises the critical question as to whether venous blood specimens are appropriate in evaluating CTCs in UM patients. UM CTCs might have been sequestered or destroyed in peripheral tissues or, alternatively, CTCs might have strong organ tropism and therefore they are repeatedly cleared from peripheral blood. The number of CTCs may also differ in different blood sources. To address these issues, we investigated the number of CTCs in paired blood specimens from both common femoral arteries and antecubital veins of the same patients with stage IV UM.

2. Patients and Methods

The protocol for blood specimen procurement was approved by the Institutional Review Board of Thomas Jefferson University. Seventeen UM patients, including ten patients who had liver only metastasis and seven patients who had hepatic and extra-hepatic metastases, were enrolled in this study between April 2014 and October 2014. All patients had treated their primary uveal melanoma between 2000 and 2013. Ten patients had received radioactive plaque as their treatment for primary uveal melanoma and seven patients had enucleation of their affected eye. None of these patients had a local recurrence of primary uveal melanoma at the time of CTC measurement. The patients signed the informed consent form prior to blood sample collection. All patients were scheduled to receive liver-directed therapy for metastatic UM. Prior to liver embolization treatment, 7.5 ml of blood samples was obtained from the common femoral arterial and the antecubital (forearm) veins, using CellSave tubes (Veridex, LLC, Raritan, NJ), and sent to the CTC measurement laboratory. The clinical information and the sources of blood were blinded to the CTC laboratory. Blood samples were maintained at room temperature and processed within 72 h of collection.

CTCs were analyzed using the standard CellSearch® protocol and CellTracks Circulating Melanoma Cell Kit® on the CellSearch® System (Janssen Diagnostics, LLC, Raritan, NJ). Briefly, cells expressing CD146 (Mel-CAM) were immunomagnetically enriched and stained with phycoerythrin (PE)-conjugated antibody specific to high molecular weight melanoma associated antigen (HMW-MAA), which is specific for melanoma cells. Allophycocyanin (APC)-conjugated anti-CD45 was used to identify leukocytes and anti-CD34 was used for detection of endothelial cells. 4,6-Diamidino-2-phenylindoledihydrochloride (DAPI) was used to detect cell nuclei. CTCs were defined as nucleated, CD146-positive cells, expressing HMW-MAA, but lacking expression of the common leukocyte antigen CD45 and CD34 endothelial markers. Samples were then scanned on the CellTracks® analyzer II fluorescent microscope (Veridex, LLC, Raritan, NJ) (Fig. 1). This technology is widespread and widely used in different counties, notably in the USA. The validity of this assay was confirmed by the Control Kit for Circulating Endothelial and Melanoma Cells provided by the manufacturer (Janssen Diagnostics, LLC). The control kit contains fixed cells from a SK-Mel-28 cell line in the bottles containing two populations of cell for high and low control. The control cells are fully compatible with CellSearch Endothelial Cell and CellTracks Circulating Melanoma Cell Kit reagents and are automatically identified by the CellTracks analyzer. The detected fixed SK-Mel-28 cells in two different lots for the studied melanoma CTC measurements were as follows: Lot #1: 1272 (mean) with the range of 1058–1486 in high control, and 62 (mean) with the range of 32 to 92 in low control; and Lot #2: mean of 1203 and the range of 719–1687 in high control, and mean of 53 and the range 11 to 95 in low control.

We also conducted exploratory experiments by adding UM cells to the healthy donor peripheral blood. In the first spiking experiment, various numbers of melanoma cell (5, 15, 30, 100, 500, and 1500 cells) obtained from a long-term cultured metastatic UM cell line (TJU-UM001) were added to 6 tubes of whole blood specimen obtained from a healthy donor. Various numbers of fixed melanoma cells were diluted in CellSearch Dilution Buffer® and then added to 7.5 ml of whole blood. The information regarding numbers of melanoma cell in individual specimens was blinded to examiners and spiked UM cells were