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Detection methods of circulating tumor cells in cutaneous melanoma: A systematic review

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Abstract

The vast majority of melanoma-related deaths are due to disseminated malignancy. Many treated patients who are clinically disease-free will go on to relapse. Therefore, new prognostic tools must be developed to better assess metastatic potential and assist in patient management. Circulating tumor cells are a widely studied metastatic biomarker with promising prognostic utility, as the shedding of cells from the primary tumor into peripheral blood is a necessary step in disease dissemination. An assortment of technologies and techniques has been developed to isolate and detect circulating melanoma cells (CMCs), but a standardized method is yet to be established. It is the aim of this study to systematically review the diverse enrichment and detection methods of circulating tumor cells in cutaneous melanoma. A literature search yielded 351 articles, of which 74 were deemed eligible according to inclusion criteria, the primary requirement being the reporting of patient CMC positivity status stratified by the stage of melanoma. Pertinent studies were used to evaluate the advantages and disadvantages of each method. Additionally, we calculated the sensitivity and specificity of seven common melanoma-associated markers based on the available literature.

Keywords: Cutaneous melanoma; Circulating tumor cells; Detection; Cancer; Systematic review

1. Introduction

Despite accounting for only 5% of all skin cancer cases, malignant melanoma is responsible for 75% of all skin cancer-related deaths [1,2]. Highly treatable in its early

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stages, the 5 year survival rate of those with localized melanoma is 98%, compared to 62% and 15% for patients with regional and distant metastasis respectively [2]. Subsequently, the vast majority of melanoma-related deaths are due to disseminated malignancy, where the median survival of metastatic patients is 6–9 months [3].

The unfavorable prognosis of advanced stage patients highlights the need for effective prognostic techniques, allowing more accurate assessment of individual susceptibility to recurrence or metastasis. Recurrence or metastasis can occur decades after the primary melanoma was excised [4–7]. Therefore, techniques that could identify metastatic potential in current patients or minimal residual disease in treated patients would be extremely valuable. Evaluation of disease potential must not only become more detailed, but also more efficient as the incidence rate of cutaneous melanoma has increased by 2.6% annually over the past 30 years [8]. A surrogate marker of metastatic melanoma that has been widely studied is the presence of circulating tumor cells (CTC) in the peripheral blood.

The shedding of cells from the primary tumor into the blood stream is a necessary but not sufficient step in the development of disseminated malignancy [9]. CTCs are found in very low concentrations in the blood and thus pose a challenge for isolation and detection [10,11]. Finding effective biomarkers is complicated by the heterogeneity of the CTC population along with the loss of tumor-associated antigens [12–16]. Although model systems predict up to 10^6 tumors cells per gram of tumor can be introduced into circulation every day [17], the majority of CTCs do not survive long in peripheral blood, frequently undergoing apoptosis [10,18]. An early experiment found that less than 1% of tumor cells survived longer than 24 h if injected into the circulation of animal models [19]. The majority of surviving cells enter a state of dormancy due to angiogenic failure or immune suppression [20,21]. Evidence of long-term melanoma dormancy is seen in lethal donor-transmitted disease following organ transplants [22–28], where recipients can develop metastasis de-novo despite the donor being clinically disease-free for up to 32 years [22,23]. Detection of CTCs could potentially allow an assessment of metastatic potential and the actual extent of subclinical malignancy depending on the presence of circulating melanoma cells, their abundance and the markers they express. Although the clinical utility of circulating melanoma cells (CMCs) is controversial, the presence and abundance of tumor markers in the blood has been correlated with advanced patient stage [29–35], decreased overall survival [30,36–40] and decreased disease-free survival [30,39,41–43]. Therefore, the effective detection and characterization of CMCs is an imperative first step toward fully understanding and utilizing this prognostic tool, as its potential for clinical use has been proven in patient studies and is grounded in its theoretical necessity for metastasis.

2. Materials and methods

A systematic review of articles on the detection techniques for CMCs was done by searching EMBASE, PubMed, MEDLINE, PreMEDLINE and HealthSTAR databases. Search terms used involved the use of the medical subject headings, “Melanoma” and “Neoplastic Cells, Circulating” along with one of the following variable search terms including “detection”,“assay”, “marker” or “method”. The titles and abstracts of all original articles published in English between January 1990 and December 2013 were screened for relevance; pertinent articles were then reviewed in detail.

Eligible studies were published in peer-reviewed journals, letters and meeting abstracts were eliminated. Primary studies with a cohort of melanoma patients were used specifically, excluding studies involving animal models or cell line spiking experiments. CMC status of the patients (positive/present or negative/absent) had to be clearly identified, along with the stage of melanoma at the time of testing. The particular technique used to detect the circulating melanoma cells also had to be described, along with the melanoma-associated biomarkers. Additional requirements included the strict use of peripheral blood for the detection of CMCs, studies isolating cells from the lymphatic system were disregarded. Only articles that evaluated detection in cutaneous melanoma were eligible, studies involving uveal melanoma were excluded if cutaneous data was not presented separately in the analysis.

Articles that met the identified inclusion criteria were analyzed in detail and the following information was recorded for each: author, year published, technique used, enrichment used prior to detection, detection method, melanoma-associated markers, number of patients, patient CMC status as well as the number of negative controls (both healthy and non-melanoma cancer patients) with corresponding CMC status. In the case of multiple samples per patient taken at a single time, the number of samples was noted along with the sample volume. However, studies that conducted serial testing without reporting baseline positivity data were excluded. In addition, evaluation of the advantages/disadvantages of a technique in studies that met inclusion criteria were also noted, if these evaluations were based on data from the detection of CMCs in melanoma patients specifically.

The computerized literature search yielded 351 records. Screening of abstracts was done for all search results, while studies deemed relevant were examined fully. After review of the search results and the references of pertinent articles, 74 studies met all inclusion criteria. The types of CMC enrichment found included erythrocyte lysis (19 of 74, 26%), density gradient centrifugation (24 of 74, 32%), immunomagnetic enrichment (8 of 74, 11%), ISET (2 of 74, 3%) and OncoQuick (1 of 74, 1.3%). The detection techniques included RT-PCR (53 of 74, 72%), qRT-PCR (12 of 74, 16%), CellSearch (4 of 74, 5%), manual immuocytometry (5 of 74, 7%), FACS (2 of 74, 3%) and photoacoustic flow cytometry (1 of 74, 1.3%). A wide variety of melanoma-associated