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Review

Liquid biopsy utility for the surveillance of cutaneous malignant melanoma patients

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ABSTRACT

Cutaneous melanoma is one of the highest incident-rate cancers with increasing prevalence in Western societies. Despite the advent of new approved therapeutics, the 5-year overall survival rate of stage IV melanoma patients remains below 15%. Current treatments for late stage disease have shown higher efficacy when treated at a lower disease burden. Thus, blood-based biomarkers capable of detecting melanoma prior to clinically evident distant metastasis, will improve the treatment and outcomes for melanoma patients. To that end, effective treatment of melanoma necessitates identification of patients at risk for developing distant metastases. Furthermore, employing blood biomarkers that monitor cancer progression over the course of treatment is a promising solution to post-treatment drug resistance often developed in melanoma patients. Non-invasive blood biomarker assays allow for regular dynamic monitoring of disease. "Liquid Biopsy" of blood, which exploits circulating tumor cells (CTCs), cell-free circulating tumor DNA (ctDNA) and cell-free circulating microRNA (cmiRNA), has been shown to detect prognostic factors for relapse in AJCC stage III and stage IV melanoma patients. Moreover, molecular characterization of CTC and analysis of various forms of ctDNA present promising potential in development of individualized therapy for melanoma patients. New approaches such as massive parallel sequencing (MPS) provide a comprehensive view of the disease progression, allowing for

Abbreviations: Ab, antibody; AIM1, absent in melanoma-1; AJCC, American Joint Committee on Cancer; BRAF, B-Raf proto-oncogene, serine/threonine kinase; CE, capillary electrophoresis; cmiRNA, cell-free circulating microRNA; ctDNA, cell-free circulating tumor DNA; ctDNAm, methylated ctDNA; CTC, circulating tumor cell; CNA, copy number aberration; CNG, copy number gain; CNL, copy number loss; CTLA-4, cytotoxic T-lymphocyte antigen-4; DFS, disease-free survival; ddPCR, droplet digital PCR; FABP7, fatty acid-binding protein-7; GalNAc-T, ganglioside GM2/GD2 glycosyltransferase; GP100, glycoprotein 100; HMW-MAA, high molecular weight-melanoma associated antigen; HR, hazard ratio; LDH, lactate dehydrogenase; LOH, loss of heterozygosity; LINEs, long interspersed nucleotide elements; MPS, massive parallel sequencing; MAA, melanoma-associated antigen; MART-1, melanoma antigen recognized by T-Cells 1; MAGE-A3, melanoma antigen family A3; MEK, mitogen-activated protein kinase kinase; MGMT, O-6-methylguanine-DNA methyltransferase; MSI, microsatellite instability; MSS, melanoma-specific survival; mt, mutation; OS, overall survival; PAX3, paired box gene 3; PD-1, programmed cell death 1; PD-L1, programmed cell death ligand 1; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse transcription PCR; RASSF1A, ras association domain family 1 isoform A; RAR-β2, retinoic acid receptor beta 2; SLN, sentinel lymph node; SNP, single-nucleotide polymorphism; TFPI2, tissue factor pathway inhibitor 2.

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the selection of therapeutic options for individual patients. With advancements of improving molecular assays, liquid biopsy analysis as a powerful, routine clinical assay for melanoma patients, is highly promising prospective.

1. Introduction

Cutaneous melanoma is derived from transformation of melanocytes; prolonged ultraviolet exposure is believed to be the major cause of melanoma development. Primary cutaneous melanoma mainly metastasizes to tumor-draining lymph nodes and then to distant organ sites. Melanoma is very aggressive, such that the primary tumor of a lesion as small as 2.5–4 mm in diameter can metastasize systemically to multiple organs in the body, resulting in very poor prognosis (Edge et al., 2010). Sadly, the 5-year overall survival (OS) rate in patients with stage IV melanoma is currently less than 15% (Edge et al., 2010). The standard drug treatment for advanced melanoma before 2011, which consisted of the alkylating agent dacarbazine, interleukin-2, high dose IL-2 (HD IL-2) and interferon-α-2b (IFN-α), showed limited benefit for OS (Balch et al., 2009; Girotti et al., 2014). In 2011, the FDA approved vemurafenib (B-Raf proto-oncogene, serine/threonine kinase (BRAF) inhibitor) and ipilimumab (cytotoxic T-lymphocyte antigen-4 (CTLA-4) inhibitor) for targeted therapies, providing a major breakthrough for metastatic melanoma treatment with extended progression-free and overall survival (Chapman et al., 2011; Hodi et al., 2010). In 2013, 2014, the FDA approved trametinib (mitogen-activated protein kinase kinase (MEK) inhibitor), and a combination of trametinib and dabrafenib (BRAF inhibitor), respectively. In late 2014, FDA approved pembrolizumab (programmed cell death ligand 1 (PD-L1) immune checkpoint inhibitor) and nivolumab (programmed cell death 1 (PD-1) immune checkpoint inhibitor). The Immune-targeted therapies such as ipilimumab, pembrolizumab and nivolumab have transformed the field of melanoma therapy (Girotti et al., 2014). However, tumors often acquire resistance to systemic treatments due to tumor heterogeneity, selection and clonal evolution. Thus, a blood-based biomarker is needed for monitoring the genetic/epigenetic alterations during treatment. Moreover, previous studies show that advanced stage patients exhibit the greatest treatment-efficacy when treated with a lower disease burden (Hodi et al., 2010; Sosman et al., 2012). Therefore, a blood test that detects melanoma with regional dissemination, prior to clinically evident distant metastasis, may help improve monitoring effectiveness of treatment and outcomes for melanoma patients. As of now, lactate dehydrogenase (LDH) is the only approved blood biomarker for metastatic melanoma patients, albeit it has limited sensitivity (Balch et al., 2009). Thus, the major challenge is the lack of an efficient biomarker for detecting disease progression at an early stage and/or for monitoring patients' therapy response during treatment.

Although tumor biopsy remains the gold standard for primary melanoma diagnosis and confirmation of metastatic disease, it has limitations for current clinical needs. Tumor biopsy procedure is invasive, not always possible to obtain, and inadequate for monitoring of primary tumor's genomic changes over time or detecting the subclinical tumor progression due to the intra- or inter-tumor heterogeneity. The "Liquid Biopsy" approach is based on the assessment of circulating tumor cells (CTCs), cell-free circulating tumor DNA (ctDNA) and circulating microRNA (cmiRNA) in patient's blood to extract the molecular information of primary tumor. Accordingly, liquid biopsy is an apt source for blood-based targeted molecular assays with potential utility for monitoring disease progression.

Identification of patients at risk for developing distant metastases is required for effective treatment of patients with late stage melanoma. Therefore, most liquid biopsy approaches in AJCC stage III and stage IV melanoma patients have focused on the detection of CTCs, ctDNA, and cmiRNA for a prognostic factor for relapse and a predictive factor in response to therapy; many of these studies had included longitudinal monitoring of patients. Our group and others have shown that CTCs, ctDNA, and cmiRNA exhibit notable prognostic value in terms of DFS and OS (Hoshimoto et al., 2012a, 2012b, 2012c; Khoja et al., 2015; Ono et al., 2015b; Stark et al., 2015). This review focuses on the significance of the assessment of CTCs, ctDNA, and cmiRNA by means of liquid biopsy, in patients with cutaneous metastatic melanoma. The key studies of our group as well as recent reports from others are presented here; the main discussion topics include the analytical systems used for isolation and detection of biomarkers in melanoma patients, frontiers of relevant technologies and future challenges to implement liquid biopsy in a clinical setting. Furthermore, this review examines the potential for the molecular characterization of CTC and the analysis of various forms of ctDNA in individualized therapy for melanoma patients. For a more comprehensive review of CTC, ctDNA and cmiRNA and their implications in cancer, we recommend previously published reviews in this field (Bertoli et al., 2015; Heitzer et al., 2015; Marzese et al., 2013; Rodic et al., 2014; Schwarzenbach et al., 2014).

2. Circulating tumor cells (CTC)

CTCs are cancer cells that have dissociated from the primary or metastatic tumor into the bloodstream. Melanoma CTCs were first detected in malignant patients by molecular techniques (Smith et al., 1991). Serving as an alternate for tumor tissue, CTCs are not only feasible for transcriptome and genomic profiling as biomarkers, but they also provide new insights into the metastatic mechanisms in melanoma,

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