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Mini-review

Future of circulating tumor cells in the melanoma clinical and research laboratory settings

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ABSTRACT

Circulating tumor cells (CTC) have become a field of interest for oncologists based on the premise that they constitute the underpinning for metastatic dissemination. The lethal nature of cancer is no longer attributed to solid tumor formation, but rather to the process of metastasis; shifting the focus of current studies towards the isolation and identification of metastatic progenitors, such as CTCs. CTCs originate from primary tumor masses that undergo morphologic and genetic alterations, which involve the release of mesenchymal-like cancer cells into the bloodstream, capable of invading nearby tissues for secondary tumor development. Cancerous cells contained in the primary tumor mass acquire the motile mesenchymal phenotype as a result of the Epithelial-to-Mesenchymal Transition, where substantial variations in protein expression and signaling pathways take place. CTCs that migrate from the primary tumor, intravasate into the systemic vasculature, are transported through the bloodstream, and invade tissues and organs suitable for secondary tumor development. While only a limited number of CTCs are viable in the bloodstream, their ability to elude the immune system, evade apoptosis and successfully metastasize at secondary tumor sites, makes CTCs promising candidates for unraveling the triggers that initiates the metastatic process. In this article, these subjects are explored in greater depth to elucidate the potential use of CTCs in the detection, disease staging and management of metastatic melanoma.

Significance: The clinical promise of CTCs to serve as a predictive tool of assessment for disease staging, drug efficacy, personalization of therapeutic agents, disease-free-duration, and overall patient survival, is limited by CTC isolation technology, CTC heterogeneity, and a need for a better understanding of CTC biology. However, the CTC field can still provide valuable insight into tumor depiction, metastatic pathway elucidation, as well as the determination of precise diagnostic and prognostic tools in disease-free survival.

Introduction

Annually 76,000 individuals in the United States are affected by melanoma, making it the fifth most common and aggressive solid malignancy [1,2]. Circulating tumor cells (CTCs) are cancerous melanocytes that dislodge from the primary tumor site, migrate through the bloodstream, and invade nearby tissues or organs developing secondary tumors. For this reason, the CTC field holds promise for advancing new treatments for melanoma by targeting CTCs. CTCs acquire archetypal mesenchymal motility necessary for metastatic spread by the loss of adhesion molecules that typically characterize their epithelial counterparts in primary solid tumors. The process by which epithelial cells in the primary tumor convert to the mesenchymal phenotype is known as the Epithelial-to-Mesenchymal Transition. As a result, the CTCs undergo genotypic and phenotypic changes leading to subsequent detachment from the primary tumor site. Mesenchymal progenitor cells with high

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metastatic potential must then surpass the migratory and invasive phases of metastasis to disseminate through the bloodstream and form secondary tumors. Upon Mesenchymal transition, CTCs first invade the dermal tissues and intravasate into blood or lymphatic vessels by squeezing through tight junctions of endothelial cells and other cells in blood or lymphatic vessels, thereby gaining access into the vasculature or lymphatic systems [3]. In the bloodstream, CTCs must withstand sheer forces, evade apoptosis-inducing signals, and elude the immune system. Metastatic tumor establishment requires CTCs to move out of circulation, or extravasate into susceptible tissues that are fit for metastatic invasion [4]. Successful extravasation requires melanoma CTC's anchoring to the vascular epithelial walls. One approach involves, melanoma CTCs interactions with neutrophils or other immune cells to facilitate attachment to vessel walls [5]. In spite of CTCs accounting for only 1–3 cells of total peripheral blood concentration in a cancer patient and showing high heterogeneity in genetic and phenotypic protein expression, their use in the research setting for the diagnosis and prognosis of cancer remains limited [6]. This review provides insight into the limitations of CTCs for clinical use, the potential utility of these cells for understanding melanoma biology, and development of therapies to prevent the metastatic spread of this disease.

Circulating melanoma cells background

Epithelial-to-mesenchymal transition

Before the onset of the migratory melanoma phenotype, melanocytic cells must undergo multiple biological changes in order to disassociate from the primary tumor, which involves the Epithelial-to-Mesenchymal Transition (EMT). As the term denotes, EMT is the trans-differentiation of polarized immotile melanocytic cells to motile mesenchymal cells [7]. EMT has been categorized into 3 main subtypes, where EMT types 1 and 2 correspond to embryonic and organ fibrosis development. Although this is a natural process associated with the generation of secondary epithelia, wound healing, and organ fibrosis, EMT type 3 takes place in neoplastic cells undergoing epigenetic changes favoring malignant clonal outgrowth [8]. Epithelial cells within the primary tumor are bound together by strong cell surface ligands, known as CDH1/Epithelial Cadherin. These proteins are joined via cytoplasmic tails attached to α-catenin and β-catenin, which are woven into the actin cytoskeleton, leading to strong intercellular adhesion, typical of epithelial cells [9,10] (Fig. 1). The epigenetic variations taking place prior to the onset of EMT are associated with the expression of vimentin, zinc-finger transcription factors Snail (SNAI1) and Slug (SNAI2), Wnt, Notch, SPARC, PAPPA, Nodal, ACTN4, Hedgehog, as well as changes in the MAPK pathway (Fig. 2) [11–20]. Additionally, the transcription factor Yin Yang 1 (YY1) is often expressed in melanoma, and while it is regulated by a low expression of BMI1, it is also accompanied by overexpression of SOX2 and OCT4 [21]. The expression of vimentin plays a crucial role orchestrating EMT. Upregulation of this intermediate filament protein, which is modulated by the Slug transcription factor, increases the expression of Axl tyrosine kinase, which in turn, contributes to the cytoskeletal changes typical of the invasive migratory mesenchymal phenotype. Primary tumor cells expressing Epithelial Cadherin (CDH1), begin to secrete EGF, FGF, TGF-β, VEGF, PDGF, and HGF (epithelial, fibroblast, transforming, vascular endothelial, platelet-derived, and hepatocyte growth factors) [7,22–26]. Upon interaction with these factors, early fibroblastic melanocytic cells start to express calcium-dependent adhesion molecules CDH5 and CDH2 (Non-Epithelial Cadherins). N-Cadherin adhesion ligands bind intercellular junctions less tightly than CDH1/Epithelial Cadherin, leading to the incremental absence of cell adhesion molecules (CAMs) involved in cell surface binding within the extracellular matrix (ECM) [4,22–25,27,28]. This leads to gradual loss of CDH1/Epithelial Cadherin expression.

The morphological change from the epithelial to the mesenchymal phenotype, as observed in protein expression, is also controlled at the transcriptional and translational level by microRNAs. Evidence demonstrates that microRNAs are largely involved in the coordination of EMT by quelling the expression of various groups of transcription factors, or otherwise acting as the functional mediators in orchestrating the mesenchymal transformation [21]. In recent studies, a novel network was found which modulates the effects of ubiquitin protein ligases, transcriptional repressors, histone methyltransferases, and three miRNAs to successfully promote metastasis [29]. In experiments conducted by the National Cancer Institute, the MiR-200 family was implicated as being a strong marker for cells that express E-cadherin, while lacking expression of vimentin [21]. The role of EMT in tumor metastasis has recently shifted in focus, where miRNA are predicted to play a more important role in the mesenchymal transition. However, this area of research is in its early stages and requires further investigation.

Although recent evidence suggests that EMT plays a minor role in the metastatic dissemination of ductal adenocarcinoma (PDAC) and secondary lung malignancies, EMT's ability to confer clonal cell chemoresitance continues to hold true [30,31]. In addition, studies evaluating the extent of melanoma metastasis in EMT inhibited cell cultures targeted the over and under expression of single transcription factors notorious for inducing or inhibiting EMT. As previously mentioned, the EMT process is orchestrated by an ample repertoire of transcription factors and epigenetic changes. Perhaps, more comprehensive studies investigating the effects of multiple transcription factors can help dissipate current obscurities on the role played by EMT in metastatic melanoma.

Intravasation

Evolving melanoma cells undergoing EMT, acquire the migratory phenotype of progenitor cells [32], detach from primary solid tumors, adhere to the basement membrane, and intravasate into the systemic vasculature via dermal invasion [4,33]. These cells are attracted and nurtured by signaling molecules, proteases, and other microenvironment factors that contribute to successful tumor angiogenesis [34,35]. Enhanced expression of Wnt5a, ACTN4, and the activation of PKC increase cytoskeletal changes that heighten migratory cell properties and promote CTC dermal invasion [19,36]. Following invasion, intravasation occurs through hematogenous (blood vessel) or lymphatic dissemination (Fig. 1). The upregulation of VEGFB, downregulation of Shb, and enhanced expression of metalloproteinase MMP-1 stimulates the invasiveness of mesenchymal progenitor cells by reducing blood perfusion and increasing hypoxia, which collectively contribute to vessel leakiness [35,37,38]. Hematogenous intravasation, whether passive or active, involves endothelial cell invasion and is contingent on blood vessel structure and tumor angiogenesis. In passive intravasation, the invading mesenchymal melanoma cell expresses low levels of CDH1/E-Cadherin and leaves the primary tumor without the use of active transport [39]. On the other hand, the activation of Rac18 enhances the expression of metastatic ligands NEDD9 and DOC-3, which contribute to actin cytoskeletal alterations, which fosters migration typical of actively-induced intravasation. Active intravasation is associated with significant epigenetic changes and increased CTC heterogeneity especially in ligand expression to promote survival under sheer stress. Actively intravasating CTCs tend to have a higher metastatic potential (Fig. 1).