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Feature Article

Nanoparticle-based sorting of circulating tumor cells by epithelial antigen expression during disease progression in an animal model

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Abstract

Circulating tumor cells (CTCs) can be used as markers for the detection, characterization, and targeted therapeutic management of cancer. We recently developed a nanoparticle-mediated approach for capture and sorting of CTCs based on their specific epithelial phenotype. In the current study, we investigate the phenotypic transition of tumor cells in an animal model and show the correlation of this transition with tumor progression. VX2 tumor cells were injected into rabbits, and CTCs were evaluated during tumor progression and correlated with computerized tomography (CT) measurements of tumor volume. The results showed a dramatic increase of CTCs during the four weeks of tumor growth. Following resection, CTC levels dropped but then rebounded, likely due to lymph node metastases. Additionally, CTCs showed a marked loss of the epithelial cell adhesion molecule (EpCAM) relative to precursor cells. In conclusion, the device accurately traces disease progression and CTC phenotypic shift in an animal model.

From the Clinical Editor: The detection of circulating tumor cells (CTCs) has been used to predict disease prognosis. In this study, the authors developed a nanoparticle-mediated platform based on microfluidics to analyze the differential expressions of epithelial cell adhesion molecule (EpCAM) on CTCs in an animal model. It was found that the loss of EpCAM correlated with disease progression. Hence, the use of this platform may be further applied in other cancer models in the future.
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Key words: Nanoparticle; Circulating tumor cells; Microfluidics; Rabbit cancer model; Cell sorting

Current cancer staging methods inadequately predict tumor prognosis and response to therapy, thus underscoring the need for new tumor characterization approaches.[1,2] The heterogeneous nature of tumors and difficulty in identifying metastases during early stages of cancer confound the ability to predict tumor prognosis and determine appropriate therapy.[3,4] A promising approach, which may serve to address these problems, is the analysis of circulating tumor cells (CTCs).

CTCs are putative precursors of metastases.[5] Specifically, they are cells which are released from the perimeter of the tumor and intravasate into the blood stream.[6] These cells then circulate until they encounter an appropriate niche, at which time they may extravasate into the surrounding tissue. Rapid division then establishes a secondary tumor, which can ultimately produce its own CTCs and continue this process.

Since CTCs are derived from primary tumors and appear to be the cells that establish metastatic sites, they can provide a wealth of information regarding specific tumor biology and the driving factors behind invasive disease.[7,8] Furthermore, numerous studies have shown that CTCs in blood samples may be used as a marker to predict survival and prognosis in metastatic cancer patients.[9,10] CTC levels appear to be correlated with disease spread,[9–11] and elevated CTCs are associated with poor prognosis and increased probability of metastasis.[12]

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Importantly, CTCs represent the biological state of the evolving disease. A significant aspect of CTC biology, which has gained considerable traction in recent years, is the loss of epithelial character accompanying cancer progression during a process known as epithelial-to-mesenchymal transition (EMT).[13] This process involves a cellular reprogramming event that causes CTCs to assume a more invasive phenotype relative to the epithelial phenotype prominent in the primary tumor.[6] As a consequence, epithelial surface markers such as EpCAM are down regulated.[14] Although the consequences of this transition are not well understood, it has been implicated in increased metastatic potential, possibly stemming from increased cell mobility due to loss of adhesion molecules.[15] Furthermore, the population of CTCs with low levels of epithelial marker expression, including EpCAM, has been shown to peak during times of disease progression as compared to treatment response.[13] Thus monitoring changing levels of epithelial markers, the most prominent of which is EpCAM, should provide valuable insight into cancer progression and metastasis.

Numerous studies have successfully captured CTCs from diverse tumor origins including lung,[16] prostate,[16] head and neck,[17] melanoma,[18] gastric and pancreatic cancers.[16,19] However, despite numerous advances in CTC capture techniques, the analysis of CTCs is still not part of routine tumor staging in clinical practice. In fact, the CellSearch system is the only existing Food and Drug Administration (FDA) cleared platform.[20] Furthermore, the vast majority of CTC detection methods simply count the absolute number of CTCs, without distinguishing between varieties of CTC subpopulations. Consequentially, potentially valuable information may be overlooked.

Recently, we developed a new approach that provides a means to capture and classify of CTCs with high sensitivity and selectivity using immunomagnetic nanoparticles captured within a novel microfluidic device.[21,22] Our CTC isolation technique depends on antibodies against EpCAM attached to magnetic nanoparticles allowing for capture using a magnetic field. This device spatially sorts CTCs on the basis of EpCAM expression, thus providing insight into differential expression of epithelial markers. EpCAM is expressed by a wide variety of epithelial tumors and is a generally accepted marker of CTCs,[16] and is one of the markers known to show a significant decrease during EMT, thus establishing it as a surrogate marker of this process.[13] Sorting cells according to EpCAM expression therefore provides a means to monitor phenotypic changes in CTCs. This device was shown to allow the profiling of CTC subpopulations with differing epithelial character in samples collected from prostate cancer patients. Here, we report the application of this device to an animal model of cancer. Using a rabbit host and the VX2 tumor model, we monitored the epithelial character of CTCs during tumor growth and following resection of the tumor. A significant change in CTC profile is observed from more epithelial to less epithelial as tumors progress. This is the first study to monitor these dynamics in an animal model of cancer.

Methods

Animal model

Experiments were performed using 6 New Zealand white rabbits weighing 2.5-3.0 kg. All animal studies were performed in accordance with the University Health Network/University of Toronto guidelines for the humane use of animals. Care, handling and maintenance of all animals used in this study were conducted in a humane manner, as per the animal care experimental protocol approved by the institutional Animal Care and Use Committee of University Health Network, University of Toronto. Male rabbits (Charles River, Wilmington, Massachusetts) were injected with 300 μL of a high-density (approximately 5×10^6/mL) cell suspension of VX2 squamous cell carcinoma of the rabbit into the thigh muscles (quadriceps). Tumor development and lymph node metastases were monitored using computed tomography (CT) images and clinical evaluation bi-weekly, and were resected 4 weeks after tumor induction. Tumors and enlarged lymph nodes were sent for pathology and immunostaining analysis. Blood samples for CTCs (2-3 mL), hematology and comprehensive biochemistry analysis were collected biweekly, pre and post-operation.

Tumor cell line propagation

The VX2 tumor cell line is maintained in small tumor pieces that are frozen at -80° C. Tumor cells were propagated by injecting 500 μL of VX2 tumor into the quadriceps of propagating rabbits (different from rabbits used for the CTC study) and were harvested after approximately 3 weeks. The harvested tumor was placed in Hanks balanced salt solution (HBSS) in a sterile 100 mL container. Prior to tumor induction in rabbits, the tumor pieces were thawed and cut into small pieces using a sterile scalpel and subsequently placed on to a 70-μm-cell strainer sitting on a 50 mL tube (BD Falcon brand). A syringe plunger was used to mince the cells and ~500 μL HBSS was used to suspend the cells in the strainer (repeated several times).

CT imaging and image analysis

CT imaging (Locus Ultra, GE Healthcare, Milwaukee, Wisconsin, USA) was performed biweekly pre and post-surgical tumor resection (80 kVp, 50 mA). All CT-based image analysis was performed using Microview (GE Healthcare, Milwaukee, Wisconsin, USA) and custom in-house program written using MATLAB (MathWorks®, Natick, Massachusetts, USA). The tumor volumes were contoured using a semi-automated threshold based method. The mean and standard deviation of the voxel signal distribution within each VOI were calculated.

Histopathological evaluation

Tumor and lymph node tissue samples were fixed in formalin after resection, embedded in paraffin blocks, cut and stained with hematoxylin and eosin (H&E) and pan-cytokeratin (AE1/AE3), the intermediate filaments of epithelial cells. All histopathology images were analyzed using ImageScope (Leica Biosystems, Wetzlar, Germany) after scanning.

Capture of cell lines

100 SKBR3 or MDA-MB-231 cells in 100 μL were incubated with 10 μL of MACS anti-EpCAM nanoparticles (130-061-101) for 10 min. Cells were then introduced into the chip at a rate of 600 μL/h for 10 min. A 200 μL PBS 1× rinse was then added followed by 100 μL of PBS-4% formaldehyde and 100 μL PBS–

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