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A physical sciences network characterization of circulating tumor cell aggregate transport

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While the presence of circulating tumor cells (CTCs) in the vasculature has been implicated in the metastatic cascade of epithelial carcinomas, new evidence has revealed a putative role of CTC aggregates as a potential form of stromal-assisted metastasis across a variety of epithelial tumor types (6). In concert, the presence of homotypic interactions among CTCs leading to aggregation occurring at sites of endothelial attachment suggest the involvement of CTC aggregates in the hematogenous dissemination of cancer. Despite compelling evidence suggesting a role for CTC aggregates in metastatic progression (28), the physics underlying CTC vascular transport including the influence of blood flow, coagulation, intercellular adhesion, and collisions with cells of the vasculature, such as red blood cells (RBCs) and endothelial cells (ECs), remains poorly understood. Better physical understanding of CTC transport and mechanobiology could enable, for example, new strategies to monitor patient response to chemotherapy (22).

Here, we demonstrate a multidisciplinary approach (29) that utilizes clinical measurements on single CTCs and CTC aggregates from a patient with breast cancer as a quantitative guide in the rational design of in vitro and in silico models to investigate the dynamics of CTC transport in the vasculature. Using the high-definition (HD) CTC assay to identify circulating tumor cells in the blood, coupled with quantitative phase microscopy (QPM) to quantify the subcellular density organization of CTCs, we profiled the biophysical properties of CTCs in a patient with breast cancer. These metrics, including geometric and density features, were used as a benchmark in the growth of breast cancer cell line-based aggregates. Cell lines of varying tumorigenic potential were utilized to model the heterogeneous “cytotypes” believed to be a basic feature of solid tumors.

Microfluidic flow assays were utilized to simulate CTC-EC interactions by quantifying rolling velocity and orthogonal (applied shear flow) displacement of the cell line aggregates on surfaces coated with E-selectin. To predict dynamical features of CTC vascular transport, a model incorporating geometric measurements of CTCs was constructed that coupled elastic collisions between RBCs and CTCs to quantify rolling velocity, orthogonal displacement, and the dependence of CTC vascular margination on single CTCs and aggregate morphology and stiffness.

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and cell line-based aggregates were statistically indistinguishable for all aggregate sizes (1–5 cells/aggregate) and cell line types (MCF7, MCF10A, BT20) investigated. These findings provided a quantitative rationale for the use of cell lines as a means to replicate the biophysical features of CTCs and CTC aggregates in in vitro models of selectin-mediated rolling. In microfluidic assays to model CTC vascular transport under applied shear, we observed an upward trend in rolling velocity as the number of cells/cluster increased. Similarly, we found that the displacement of CTC aggregates in the direction orthogonal to the applied shear was increased among 4- and 5-cell aggregates in comparison with single cells. These results are consistent with predictions from the numerical simulations with increased, orthogonal-to-flow motion among aggregates with increased aspect ratios. Further, it was found that interactions of CTCs with the vessel wall were reduced as cell membrane elasticity increased. Together, these results demonstrate that an approach rooted in modeling a clinically relevant sample can provide essential guidance to in vitro and in silico methods to understand the physics of CTC vascular transport.

MATERIALS AND METHODS

Noninterferometric Quantitative Phase Microscopy (NIQPM): Transport of Intensity Equation-Based Measurement of Subcellular Density

Cellular specimens appear semitransparent when imaged under standard bright-field microscopy (14, 27). This low endogenous contrast is a result of the amplitude of the waves travelling through the cell remaining relatively unchanged owing to weak scattering and absorption over the visible wavelength range. Interestingly, the thickness and spatially variable density of the cell give rise to appreciable phase lag of the transmitted waves through the specimen. This fact has inspired the utilization of phase as a contrast mechanism in cellular imaging in modalities such as phase-contrast microscopy and differential interference contrast (DIC) microscopy (14).

Modern optical engineering approaches, including the transport of intensity equation (TIE) method, have enabled the quantification of the phase, denoted \( \phi \) [radian], of transmitted waves through cellular specimens (26). This is crucial, as knowledge of the phase enables the enumeration of the projected mass density, defined as the sum of the three dimensional density along the optical axis of the microscope, through the following relationship:

\[
\rho(x, y) = \frac{\lambda \phi(x, y)}{2\pi \alpha} \text{ (pg/\mu m}^2) \tag{1}
\]

Here \( \rho(x, y) \) denotes the projected mass density, \( \lambda \) is the wavelength of light used when determining the phase, and \( \alpha \) is the specific refraction increment of the cell solids (~0.2 ml/g). The total cellular dry mass can be determined by summing up the projected mass density over the area of the cell. In this investigation we quantified cellular organization by binning the mass density map into histograms to evaluate the mean dry mass density, standard deviation, and coefficient of variation (8).

Circulating Tumor Cell (CTC) Identification and Characterization

CTCs were identified in a peripheral blood draw from a patient who presented with a malignant breast tumor determined to be ER/PR/Her2 negative. The patient provided informed consent at Scripps Clinic (La Jolla, CA) as approved by the Institutional Review Board. Eight milliliters of peripheral blood was collected in a rare cell blood collection tube (Streck, Omaha, NE) and processed within 24 h after phlebotomy. HD-CTCs were identified according to the published protocol (20). The assay consists of a RBC lysis, after which nucleated cells are attached as a monolayer to custom-made glass slides. Slides were subsequently incubated with antibodies against cytokeratins (CK) 1, 4–8, 10, 13, and 19 (Sigma), and CD45 with Alexa 647-conjugated secondary antibody (Sero-tec); nuclei were counterstained with DAPI (Sero-tec). Automated digital microscopy was used for fluorescence imaging. Potential CTCs were located and identified by computational analysis of the resulting image data. Fluorescence images of CTC candidates were then presented to a hematopathologist-trained technical analyst for interpretation. Cells were classified as HD-CTCs if they were CK-positive, CD45-negative, contained an intact DAPI nucleus without identifiable apoptotic changes or a disrupted appearance, and were morphologically distinct from surrounding leukocytes.

Multicellular Breast Cancer Cell Line Aggregate Cultures

A lithography mask was designed using AutoCAD LT 2008 (Autodesk) (12). The mask included arrays of rectangular trenches with 100-μm circular openings. The trenches were 150 μm in depth. Silicon wafers were produced from this mask (MEMS and Nanotechnology Exchange) using the Bosch deep reactive ion etching (DRIE) process. The hydrophobic coating produced during the Bosch process was left intact. The DRIE silicon wafers were used to produce microbubbles (MB) in polydimethylsiloxane (PDMS). Dow Corning's Silgard 184 silicone elastomer kit in a 10:1 base to curing agent ratio (wt/wt) was manually mixed in a 50-ml tube for 2 min and poured onto silicon wafers. The mixture was allowed to spread out on the wafer for 30 min at room temperature and cured at 100°C for 2 h. The PDMS was then peeled off from the wafers, and the arrays were cut into small chips to be used in cell culture experiments.

Small chips containing arrays of MB were rinsed in 70% ethanol and distilled water and dried with nitrogen gas. The backside of each chip was rendered hydrophilic by etching them in a plasma chamber with atmospheric air for 10 min to keep the chips submerged in cell culture media. The chips were then blocked in 1% BSA and subjected to negative pressure in a vacuum chamber to remove the air trapped inside the microbubbles during the curing process. About 0.1 × 10^6 cancer cells (BT20, MCF7, and MCF10A) in 200 μl of cell culture media were applied to the top of the chip and incubated for 10–15 min.

The chips were rinsed twice and then placed inside a single well of a 24-well plate filled with 1 ml of cell culture media. The 24-well plate containing the MB chips was placed inside a humidified cell culture chamber maintained at 37°C with 5% CO_2 for 2 days to allow for cell cluster culture.

To construct coculture clusters to mimic tumor heterogeneity, 0.1 × 10^6 cancer cells applied on the top were composed of BT20, MCF7, and MCF10A cells in 1:1:1 ratio. The cultured tumor cell aggregates were harvested by inverting the chips in a 6-well plate containing cell-culture media and placing the plate in ultrasonic cleaner for 60 s to allow gentle agitation to remove cultured tumor cell aggregates out of the cell culture platform. The media was then collected from the 6-well plate and centrifuged at 2,000 rpm for 10 min. The resulting spheroid pellets were then dissociated in PBS with calcium and magnesium ions for rolling experiments on E-selectin coated microtubes. Seeding cells in the microbubble cell culture platform in this manner yielded an average seeding density of 10–15 cells per microbubble. The seeding density was maintained and incubation time was reduced to 1 min to seed 1–5 cells per microbubble for comparing the physical similarity of clinically obtained CTCs and CTC aggregates with the cultured tumor cell aggregates.

To determine the interaction of breast cancer CTC clusters with E-selectin, microrenathane microtubes (inner diameter of 300 μm) were functionalized with recombinant human E-selectin to mimic the inner surface of the blood vessels (5). Briefly, 50-cm-long microrenathane microtubes with 300-μm inner diameter (Braintree Scientific, MRE040) were first washed with 1X PBS and then coated with 10
µg/ml of recombinant protein G (Calbiochem, 539303) for 60 min at RT. They were then incubated with 20 µg/ml of recombinant human E-selectin/Fc-chimera for 120 min. The surface was then blocked with 5% milk (EMD Millipore, 6250) for 60 min to avoid any nonspecific interaction of the surface of the tube. The tubes were washed with PBS++ (PBS saturated with calcium chloride) (Invitrogen, 14040-133) after which harvested CTC clusters were perfused through tubes at 1 dyn/cm² using a syringe pump (KDS 230, IITC Life Sciences). Videos of rolling cells were analyzed using ImageJ (NIH) stack tools to determine rolling velocity.

Circular Microchannel Cell Adhesion Assay

Microfluidic channels of circular cross-section were fabricated in PDMS (12). Briefly, PDMS was poured around a straight metal wire of diameter 50 µm and allowed to cure. Then removal of the wire produced a smooth channel of circular cross-section and inner diameter 50 µm. The channels were incubated with a 10 µg/ml solution of protein G in 1X DPBS for 1 h and 5 µg/mL E-Selectin in PBS for 2 h and blocked with 5% milk for 1 h. The microchannels were then perfused with 1X DPBS with Ca²⁺ and Mg²⁺ prior to use. Microchannels were placed on the stage of an Olympus IX81 inverted microscope (Olympus America, Melville, NY) linked to a monitor and Sony DVD Recorder DVO-1000MD (Sony Electronics, San Diego, CA). Small clusters of COLO205 colorectal cancer cells were perfused through the microchannel system using a computer-controlled syringe pump (New Era Pump Systems) and recorded for offline velocity calculation.

Fluid Dynamic Modeling of CTC Transport

Table 1 provides an overview of the free parameters used in our numerical models.

Whole blood simulation. The fluid-structure interaction (FSI) between blood plasma and cells (RBCs and CTCs) is modeled using the incompressible Navier-Stokes equation, solved through the immersed finite element method (IFEM) (18, 19, 32):

\[
\rho \left( \frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right) - \nabla \sigma = \mathbf{f}_{\text{FSI}} + \mathbf{f}_{\text{ext}} \quad \text{in } \Omega, \tag{2}
\]

\[
\nabla \cdot \mathbf{u} = 0 \quad \text{in } \Omega, \tag{3}
\]

\[
\mathbf{f}_{\text{FSI}} = \begin{cases} (\rho_{i} - \rho_{p}) \left( \frac{\partial \mathbf{u}}{\partial t} + \mathbf{u} \cdot \nabla \mathbf{u} \right) \\ + \nabla \sigma^{x} - \nabla \mathbf{u}^{x} + \mathbf{f}_{\text{ext}}^{x} - \mathbf{f}_{\text{ext}}^{x} \quad \text{in } \Omega_{s} \\ 0 \quad \text{in } \Omega_{t} \end{cases} \tag{4}
\]

where \( \rho_{p} \) is the blood plasma density, \( \mathbf{u} \) is the velocity vector, \( t \) is the time, \( \mathbf{f}_{\text{FSI}} \) is the FSI force based on the entire domain (\( \Omega \)) including both fluid (\( \Omega_{f} \)) and solid (\( \Omega_{s} \)), and \( \mathbf{f}_{\text{ext}}^{x} \) is the external forces acting on the surface of the solid domain except for the FSI force by fluid flow. In this simulation, cellular interactions between RBCs and CTCs are treated as the external force. The solid stress \( (\sigma^{x}) \) in Eq. 3 is calculated by assigning RBCs as hyperelastic material and CTCs as linear-elastic material. When the CTC is rigid, the solid stress term goes to zero.

Circulating tumor cell (CTC) modeling. The elastic features of CTCs were modeled using results from atomic force microscopy based measurements of the Young’s modulus, \( E \), reported by Cross et al. (7) for cancer cells identified in body cavity pleural effusions. Using the experimental data, CTCs are numerically constructed as a linear-elastic material through:

\[
\sigma^{x} = E \varepsilon^{x} \tag{6}
\]

where the CTC elasticity \( E \) is assumed as a value between 0.5 and 1.0 kPa. The strain \( (\varepsilon^{x}) \) is obtained from the CTC deformation at each time step.

RBC-CTC and RBC-RBC interactions. The cellular interactions between cells are assigned by using a Morse-type potential defined as

\[
\Phi_{M}(r) = D_{0} \left( e^{2\beta(r-\delta)} - 2e^{\beta(r-\delta)} \right), \tag{7}
\]

where \( r \) is the distance between cell surfaces, \( D_{0} \) is the magnitude of the interaction potential, \( \beta \) is the scaling parameter, and \( r_{0} \) is the critical length.

Statistical Analysis

A paired t-test was used for statistically analyzing the data using cell lines. For the analysis of the physical parameters of CTCs from patients, we utilized a nonparametric analog of the standard analysis of variance, the Kruskal-Wallis test, to assess statistical significance among the data. Bonferonni post hoc correction was used to eliminate familywise errors across multiple parameter comparisons. \( P \) values of 0.05 or less were considered significant.

RESULTS

Quantitative Imaging to Biophysically Profile CTC and Cell Line-Based Aggregates

An essential feature of in vitro models of the vascular transport of CTCs is the accurate representation of CTC physical properties among the cellular constituents of the model system. We first defined the physical parameters of single CTCs and CTC aggregates from a single clinical sample in order to provide a reference for the development of an in situ model of cultured tumor cell aggregate transport. We performed NIQPM imaging to both image (Fig. 1) and quantify (Fig. 2) the subcellular organization of dry mass density of CTCs and cultured tumor cells, respectively. Organizational features were quantified through the mean density, standard deviation of the density, coefficient of variation, and the total dry mass content. In parallel, DIC microscopy was performed (Fig. 1) to quantify geometrical features including area, perimeter—the derived radii from these quantities—aspect ratio, and

Table 1. Parameters for incompressible Navier-Stokes CTC transport simulations

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood plasma density</td>
<td>1,000.0 kg/m³</td>
</tr>
<tr>
<td>Blood plasma viscosity</td>
<td>1.2 × 10⁻³ Pa·s</td>
</tr>
<tr>
<td>RBC cytoplasm density</td>
<td>1000.0 kg/m³</td>
</tr>
<tr>
<td>RBC cytoplasm viscosity</td>
<td>6.0 × 10⁻³ Pa·s</td>
</tr>
<tr>
<td>( C_{1} ) for RBC hyperelastic material</td>
<td>20 Pa</td>
</tr>
<tr>
<td>( C_{2} ) for RBC hyperelastic material</td>
<td>1.0 Pa</td>
</tr>
<tr>
<td>( D_{s} ) for RBC-RBC intracellular interaction</td>
<td>1.0 J/m²</td>
</tr>
<tr>
<td>( r_{0} ) for RBC-RBC intracellular interaction</td>
<td>0.49 μm</td>
</tr>
<tr>
<td>( \beta ) for RBC-RBC intracellular interaction</td>
<td>3.84 µm⁻¹</td>
</tr>
<tr>
<td>( D_{s} ) for CTC-RBC intracellular interaction</td>
<td>3.0 J/m²</td>
</tr>
<tr>
<td>( r_{0} ) for CTC-RBC intracellular interaction</td>
<td>0.49 μm</td>
</tr>
<tr>
<td>( \beta ) for CTC-RBC intracellular interaction</td>
<td>3.84 µm⁻¹</td>
</tr>
</tbody>
</table>

CTC, circulating tumor cell; RBC, red blood cell. See MATERIALS AND METHODS for parameter definitions.
Fig. 1. Mass density imaging of CTC and cancer cell line aggregates. Differential interference contrast (DIC) images of clusters consisting of 1–5 indicated cells are presented along non-interferometric quantitative phase (NIQPM)-based imagery of the projected mass density. Representative images of a CTC cluster isolated from a single breast cancer patient are shown (CTC, top panel). Coculture was performed by mixing BT-20, MCF-7, and MCF10A cells at 1:1:1 ratio (Co-culture, bottom panel).
eccentricity (Fig. 3). All biophysical metrics were explored as the number of cells per aggregate ranged from 1 to 5.

We found that the total mass of both CTC and cultured cell line aggregates increased linearly in the same manner as a function of the number of cells per aggregate (Fig. 2A). Further, we found that all subcellular density features were conserved among CTCs and cell line aggregates, independent of the number of cells per aggregate (Fig. 2, B–D).

Geometrically, equivalent values for CTCs and cultured cancer cell aggregates were observed for the physical parameters of mean area for each aggregate ranging in size from 1 to 5 cells/aggregate, effective radius determined from area, mean perimeter for each aggregate, and effective radius determined from the perimeter (Fig. 3E). In contrast, singlet CTCs exhibited an increase in aspect ratio and eccentricity compared with single cultured cancer cells, demonstrating that singlet CTCs from this patient more closely resemble ellipses while their cell line counterparts more closely resemble circles (Fig. 2). Note that circles are characterized by an aspect ratio of 1 and eccentricity of 0.

In Vitro Modeling: Microfluidic Investigation of CTC Vascular Transport

Having quantitatively validated the use of breast cancer cell line models to simulate CTC aggregates found in a patient sample, we next used a microfluidic flow assay (5, 10) to explore hemodynamic forces on cancer cell cluster transport. We first characterized rolling velocity on E-selectin as a function of cell line type (Fig. 4) and then as a function of cell line aggregate size (Fig. 5). To elucidate cell line dependency of rolling velocity under hemodynamic forces, aggregates were placed in functionalized capillary tubes coated with 20 μg/ml E-selectin and then subjected to shear flow (Fig. 4B). E-selectin functionalized microtubes have been used previously to study the interaction of cancer cells with E-selectin at physiologically relevant flow rates. The shear stress exerted by blood in capillaries and venules is on the order of 1 dyn/cm² and higher, as used in our flow experiments. It has been shown that an E-selectin density of 20 μg/ml produces rolling behavior that is comparable to leukocyte and tumor cell rolling dynamics in mouse cremaster muscle venules and on stimulated endothelial cell monolayers (9, 15). As shown in representative rolling velocity profiles (Fig. 4C), the cell line aggregates displayed irregular rolling velocities as a function of time indicating the presence of “catch-slip” dynamics though selectin-mediated tethering. Complete arrest was observed among BT20 cell aggregates on the order of tens of seconds while a nearly constant rolling velocity with 3% fluctuations was observed for MCF7 cells. We performed temporal averaging of the velocity profiles to quantify the mean rolling velocity of each cell line (Fig. 4D). We also calculated the number of rotations per minute for BT20, MCF7, and cocultured cell line aggregates using post-processing plug-ins in ImageJ (Fig. 4D). Briefly, a pixel marker was identified on the perimeter of each cell line aggregate and the position of the marker was monitored with time. The rolling velocity (plotted on the right y-axis of Fig. 4D) of MCF7 cells (a weakly metastatic breast cancer cell line) was higher than BT20 (a highly metastatic breast cancer cell line). The average rolling velocity of cocultured clusters generated using breast cancer cell lines BT20 and MCF7 and the nontumorigenic epithelial cell line MCF10A was lower than MCF7 aggregates but higher than BT20 aggregates, indicating a synergistic effect of each cell line within the coculture aggregate to the rolling velocity profile on E-selectin coated microtubes. The average number of rotations per minute (plotted to correspond with the left axis of Fig. 4D) was higher for MCF7 aggregates than BT20 aggregates, suggesting strong interaction of BT20 aggregates with E-selectin. The average
number of rotations per minute for cocultured clusters was higher than BT20 but lower than MCF7 aggregates indicating the effect of each of the cell types in the cocultured aggregates on their interaction with E-selectin. Comparing the trend in translational rolling velocity and the average number of rotations per minute for each of the aggregate cell type indicates that BT20 cell aggregates have a significantly stronger interaction with E-selectin under flow when compared MCF7 cell aggregates. The cocultured aggregates have a stronger interaction than MCF7 cell aggregates but weaker interaction than BT20 cell aggregates owing to the contribution of each of the cell type in the cocultured aggregate to their adhesive characteristics on E-selectin coated microtubes.

Next, we investigated the geometric dependence of cluster dynamics under shear flow. Using live cell imaging, aggregates composed of COLO205 colorectal cancer cells were exposed to shear flow and their rolling velocity measured (Fig. 5A). We observed an upward trend in rolling velocity as the number of cells in the aggregates increased (Fig. 5B). Additionally, the trajectory of the aggregate was recorded as a function of time; representative profiles that demonstrate the heterogeneous nature of the rolling dynamics of the aggregates are shown in Fig. 5C. Temporal averages of the orthogonal displacement were determined (Fig. 5D).

In Silico Modeling: Numerical Investigation of CTC Vascular Transport

The CTC dynamics in the microvasculature is modeled by the immersed finite element method (IFEM) that simulates whole blood including plasma, CTCs, and RBCs. Figure 6A shows details on the computational domain. A no-slip boundary condition is prescribed on the circumferential wall of the microvessel. The velocity at the inlet of the microvessel is given as a parabolic profile with a maximum velocity of 100 μm/s. The blood plasma in the microvessel is modeled as a fluid with a density of 1,000 kg/m³ and a viscosity of 0.0012 Pa·s. The diameter and thickness of RBCs are 7.84 and 2.56 μm, respectively. The RBCs are deformed by a hyperelastic material description with two material constants, $C_1 = 20$ Pa and $C_2 = 1.0$ Pa. The cytoplasm inside the RBC membrane is described as a fluid that has a density of 1,000 kg/m³ and a viscosity of 0.006 Pa·s. CTCs are modeled as a sphere with a diameter ranging from 7 to 10 μm. Representative images of
the simulated CTC and RBC dynamics within the microvessels are presented in Fig. 6B.

While it is known that flow-dependent interactions with RBCs in the vessels of the microcirculation give rise to the prevalence of less dense cells along the periphery of the vessel wall (e.g., platelets, leukocytes), a phenomenon known as margination, the role of membrane elasticity in this phenomena is less understood. Figure 6C shows the stiffness effect of CTCs in the microvessel. For this parametric study we fixed the single CTC size to a 7-μm diameter. In the simulations we explore three regimes of CTC elasticity: rigid body dynamics, a linear elastic of $E = 1.0 \text{kPa}$, and a linear elastic with $E = 0.5 \text{kPa}$. We observed that in the rigid CTC model, single CTCs are directed towards the wall quickest compared with both the linear elastic models of CTCs. The softest CTC, with $E = 0.5 \text{kPa}$, fluctuates along its trajectory in the microvessel for the entire time of the computation. The numerical model suggests that single CTCs with more rigid membranes marginate quicker than those with softer membranes, indicating that deformation of the membrane during collisions with RBCs can prolong the time in which CTCs are transported by blood flow.

Finally, the effect of CTCs aggregates were investigated using IFEM. Singlet CTC exhibited straight-line motion under parabolic capillary flow conditions (Fig. 7, A and B) while CTC aggregates with pronounced aspect ratios rotated and tumbled during flow (Fig. 7, A and B). We quantified the mean displacement magnitude by temporally averaging the displacements, examples of which are presented in Fig. 7C. Significant displacement alterations were observed when comparing linear/triangular CTC aggregates to single CTCs.

**DISCUSSION**

It is known that there is a steady-state distribution of CTCs in the vascular system of many patients, yet it is unclear how these cells drive or participate in metastatic growth, contributing to ~90% of cancer-related deaths. This highlights the importance of understanding the underlying physical biology of, and strategies to disrupt, CTC vascular transport. Importantly, several microfluidics platforms have been developed to gain better insights into the biomechanics of the complex metastatic cascade at a cellular and molecular level with...
enormous potential for the improvement of targeted therapeutic applications (3, 17, 30, 31). Despite these recent technical advances, the influence of CTC biophysical properties on translational, rotational, inducement of hemodynamic deformation, and margination of CTCs during their transport through the vasculature has remained enigmatic. These features are crucial in understanding the relative contribution of fluid dynamics on the dissemination of cancer to secondary sites.

Here, we report an interdisciplinary approach to translate measurements of CTCs derived from a patient sample for in vitro and in silico studies of CTC transport. Using the HD-CTC assay to identify CTCs in the blood of a breast cancer patient, we quantified the features of these cells to provide a clinically relevant biophysical benchmark for both microfluidic and computational exploration of CTC vascular transport. Specifically, through the use of label-free QPM we quantified the geometric and density features of CTCs and used these quantities as quantitative thresholds for the development of breast cancer cell line-based aggregates. Cell lines of varying tumorigenic potential were utilized to model the heterogeneous “cytotypes” believed to be a basic feature of solid tumors. Because this study was carried out only on a single patient with breast cancer, it remains to be established how representative the identified cellular characteristics will be in a larger sample set.

QPM imagery revealed that both the geometric (area, perimeter, aspect ratio, eccentricity) and subcellular density organization (mass, mean density, fluctuations about the mean, coefficient of variation) of CTC aggregates and cell line-based aggregates were statistically indistinguishable for all aggregate sizes (1–5 cells/aggregate) and cell line types (MCF7, MCF10A, BT20) investigated. These findings provided a quantitative rationale for the use of cell lines as a means to replicate the biophysical features of CTC aggregates in in vitro models of cancer cell recruitment, rolling, and adhesion to the endothelium under physiologically relevant shear flow conditions.

To investigate tumor cell adhesion in the microcirculation (11, 21), microfluidic flow assays were utilized to simulate CTC-EC interactions by quantifying rolling velocity and displacement of the cell line aggregates on E-selectin coated surfaces. The selectin-mediated capture of leukocytes (e.g., neutrophils) from flowing blood has been extensively studied in flow systems of various designs (24). Red blood cells influence the distribution of neutrophils in the flow stream and, thus, contact with the vessel wall, through a process termed margination. Margination is caused by red blood cell aggregation and the tendency for red blood cells to migrate toward the centerline, thereby displacing leukocytes and platelets (or, in concept, circulating tumor cells of similar dimensions and deformability) toward the wall. Margination and leukocyte-
wall contact have been found to increase at slower flow rates and for conditions that promote red blood cell aggregation (13, 25). Munn et al. (23) showed that the addition of red blood cells to a lymphocyte suspension promoted adhesion of the lymphocytes to endothelial cells even at low hematocrit. Abbitt and Nash (1) showed that the adhesion of isolated neutrophils to P-selectin coatings was negligible when flowing through vertical tubes; however, when flowing in whole blood, many neutrophils were observed to adhere at equivalent volumetric flow rates. In a later work, Abbitt and Nash systematically

Fig. 6. Dependence of CTC margination on cell membrane stiffness. A: schematic representation of the computational domain. B: representative snapshots of the simulated dynamics of CTC and RBCs within the microvessel. C: quantification of CTC margination, via numerical tracking of distance from vessel wall, as a function of membrane elasticity.

Fig. 7. Fluid dynamic modeling of aggregate motion orthogonal to fluid flow. A: snapshots of singlet, and 4-cell aggregates with either linear or triangular geometry. B: computed trajectories of aggregates under shear flow; direction of flow is left to right. C: mean displacement magnitude for each aggregate type. *P < 0.05 with respect to linear aggregates, **P < 0.05 with respect to triangular aggregates.
varied the hematocrit from 10 to 50%, showing an increase in the degree of leukocyte adhesion to P-selectin coated glass capillaries (2). This effect was found to become insensitive to hematocrit at values above 30%. Once wall contact is made, leukocytes can interact and adhere with the endothelium in a manner that agrees remarkably well with in vitro and in silico systems that neglect the presence of the red blood cells that first brought them to the vessel wall region (15, 16). In the current study, we found that cancer cells exhibited an upward trend in rolling velocity as the number of cancer cells/aggregate increased. Similarly, we found that the displacement of cancer cell aggregates in the direction orthogonal to the applied shear was increased among aggregates consisting of 4–5 cells compared with single cells. It is worth mentioning that CTC(s) recruitment and adhesion to the vascular endothelium can also be enhanced by activated platelets through secondary tethering mechanisms (4).

To characterize the elastodynamics of CTC vascular transport, a model incorporating geometric measurements of CTCs was constructed that coupled elastic collisions between RBCs and CTCs to quantify rolling velocity, orthogonal displacement, and the dependence of CTC vascular margination on CTC morphology and stiffness. We found that CTCs with more rigid membranes marginate quicker than those with softer membranes, indicating that deformation of the membrane during collisions with RBCs can prolong the time in which CTCs are transported by blood flow.

With the recent ability to both image and quantify CTC structure, this study addresses the unmet need to rationally design culture-based and computational models to investigate the hematogenous time-force journey of cancer.

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DISCLOSURES

P. Kuhn and K. Bethel have ownership in Epic Sciences, which has licensed the HD-CTC technology. No conflicts of interest, financial or otherwise, are reported.

AUTHOR CONTRIBUTIONS


REFERENCES


