Direct Measurements on CD24-Mediated Rolling of Human Breast Cancer MCF-7 Cells on E-Selectin

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S Supporting Information

ABSTRACT: Tumor cell rolling on the endothelium plays a key role in the initial steps of cancer metastasis, i.e., extravasation of circulating tumor cells (CTCs). Identification of the ligands that induce the rolling of cells is thus critical to understanding how cancers metastasize. We have previously demonstrated that MCF-7 cells, human breast cancer cells, exhibit the rolling response selectively on E-selectin-immobilized surfaces. However, the ligand that induces rolling of MCF-7 cells on E-selectin has not yet been identified, as these cells lack commonly known E-selectin ligands. Here we report, for the first time to our knowledge, a set of quantitative and direct evidence demonstrating that CD24 expressed on MCF-7 cell membranes is responsible for rolling of the cells on

E-selectin. The binding kinetics between CD24 and E-selectin was directly measured using surface plasmon resonance (SPR), which revealed that CD24 has a binding affinity against E-selectin (K_D = 3.4 ± 0.7 nM). The involvement of CD24 in MCF-7 cell rolling was confirmed by the rolling behavior that was completely blocked when cells were treated with anti-CD24. A simulated study by flowing microspheres coated with CD24 onto E-selectin-immobilized surfaces further revealed that the binding is Ca^{2+}-dependent. Additionally, we have found that actin filaments are involved in the CD24-mediated cell rolling, as observed by the decreased rolling velocities of the MCF-7 cells upon treatment with cytochalasin D (an inhibitor of actin-filament dynamics) and the stationary binding of CD24-coated microspheres (the lack of actins) on the E-selectin-immobilized slides. Given that CD24 is known to be directly related to enhanced invasiveness of cancer cells, our results imply that CD24-based cell rolling on E-selectin mediates, at least partially, cancer cell extravasation, resulting in metastasis.
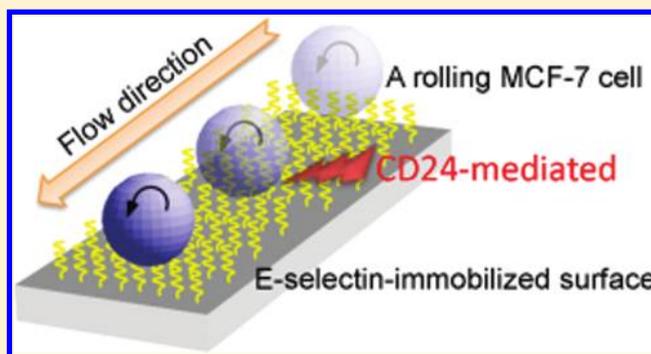
A dhesive interactions between tumor cells and the vascular endothelium are necessary to promote cancer metastasis induced by circulating tumor cells (CTCs).[1−3] The transient adhesion of cells to the endothelium in blood circulation, known as cell rolling, is the early event of the adhesive interactions that are mediated by adhesive proteins such as selectins on the endothelium and specific ligands on the cancer cell surface.[4−6] To date, three types of selectins have been identified, E-selectin (CD62E), L-selectin (CD62L), and P-selectin (CD62P).[7] Among them, E-selectin, an inducible endothelial cell-surface glycoprotein,[8] has been suggested as one of the major receptors responsible for causing the metastasis of primary tumors such as breast,[9] colon,[4] and prostate cancers.[10,11] E-selectin has been also shown to mediate the adhesion of leukocytes or carcinoma cells to cytokine-activated human endothelial cells.[12−14] Information regarding the ligands for E-selectin on the cancer cells would thus be crucial to understand the mechanism of metastasis and to potentially find a way to prohibit the spreading of primary cancers in patients.

Earlier reports have shown high-affinity binding between E-selectin and its ligands such as E-selectin ligand-1 (ESL-1),[15]

P-selectin glycoprotein ligand-1 (PSGL-1),[16,17] and sialyl Lewis X (sLe^x) and related sulfated structures including sialyl Lewis^a (sLe^a).[18−20] In addition to these known ligands for E-selectin, it is reported that the following molecules also have binding affinity to E-selectin; CD43,[21] hematopoietic cell E- and L-selectin ligand (HCELL; a specialized glycoform of CD44),[12] β2 integrins,[22] and CD44v4.[23] In our previous report, MCF-7 cells exhibited the rolling behavior only on E-selectin-immobilized surfaces under flow but no interactions with P-selectin.[24] However, to the best of our knowledge, a ligand against E-selectin present on MCF-7 cells has not been identified since MCF-7 cells lack all the known E-selectin ligands listed above.[23,25,26]

CD24, which is highly expressed by MCF-7 cells, is a mucin-like glycosylphosphatidyl-inositol-linked cell-surface protein consisting of 27 amino acids.[27] Expression of CD24 from MCF-7 cells was assessed using a fluorescence-activated cell

sorter (FACS) as shown in Supporting Information Figure 1. This protein is also expressed by early stage B-cells and neutrophils, whereas it is not expressed by normal T-cells and monocytes.[27] Previous reports have also shown that it is highly expressed on several nonhematopoietic tumors such as breast carcinomas, epithelial ovarian cancer, small and nonsmall cell lung carcinoma, prostate cancer, pancreatic cancer, and renal cell carcinoma.[11,28,29] A higher expression level of CD24 on cells in cancer patients has been correlated with a decreased survival rate, and thus CD24 has demonstrated potential to be used as a marker for more aggressive cancers.[28,30] In cancer cell lines, the expression of CD24 molecules is known to enhance the metastatic properties of carcinoma cells, e.g., the adhesiveness[29,30] and invasion in vitro[31] and in vivo.[32,33] Moreover, expedited cell growth and metastatic properties of MCF-7 cells were inhibited by cross-linking of CD24 using antihuman CD24 rabbit polyclonal antibodies on the cell surface.[34] On the basis of the in vitro and in vivo connection between CD24 and the cancer cell properties as well as our previous observation of MCF-7 cell rolling, the possibility that CD24 may be a ligand for E-selectin has emerged.

In this study, we hypothesize that CD24 is a ligand for E-selectin, which mediates cell rolling. To test the hypothesis, the binding kinetics of CD24 with E-selectin were measured directly and quantitatively using surface plasmon resonance (SPR) provided by Biacore technology. We have also found that the rolling response of the CD24-positive MCF-7 cells was completely blocked by anti-CD24 pretreatment to the cells. Additionally, we have tested the rolling velocities of MCF-7 cells treated with cytochalasin D (cytoD), an actin-disrupting agent, revealing that the cellular mechanics, such as actin-filament dynamics, play a role in the rolling mechanism mediated by CD24 and E-selectin. The involvement of the actin filament was further supported by the observed no rolling but stationary binding of CD24-coated microspheres on E-selectin. Our results presented here support our hypothesis that CD24 is a ligand for E-selectin and induces the rolling of the MCF-7 cells.

■ EXPERIMENTAL SECTION

Materials. Recombinant human E-selectin/Fc chimera (E-selectin) and antihuman epithelial cell adhesion molecule (EpCAM)/TROP1 antibody (anti-EpCAM) were purchased from R&D Systems (Minneapolis, MN). Recombinant human CD24 and sLe^x–PAA–biotin were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA) and Glycotech (Gaithersburg, MD), respectively. Unconjugated goat antihuman IgG (H + L) was acquired from Pierce Biotechnology, Inc. (Rockford, IL). The epoxy-functionalized glass surfaces (SuperEpoxy2) were purchased from TeleChem International, Inc. (Sunnyvale, CA). PE mouse IgG2a (k isotype control), PE mouse antihuman CD24, and purified mouse antihuman CD24 were obtained from BD Pharmingen (BD Biosciences, San Jose, CA). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO), unless otherwise specified, and used without further purification.

Surface Plasmon Resonance Measurements. A Biacore X instrument (GE Healthcare, Pittsburgh, PA) was used for quantitative and direct measurements of the binding kinetics of CD24 and sLe^x with E-selectin at 25 °C. The E-selectin was immobilized on CM5 sensor chips (GE Healthcare) using the amine coupling kit (GE Healthcare) as described earlier (Supporting Information Figure 3).[35] Briefly, 6 μL of E-selectin solution (1 mg/mL in PBS buffer) was diluted in 144 μL of sodium acetate buffer (immobilization buffer, 10 mM, pH 5.0). The protein solution (70 μL) was then injected into the channel 2 of a sensor chip CM5 that was preactivated by 70 μL of a mixture of 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) and N-hydroxylsuccinimide (NHS). After protein immobilization, the remaining reactive ester residues were blocked by the injection of 70 μL of ethanolamine (pH 8.5). The whole protein immobilization process was carried out at a flow rate of 5 μL/min using HBS-EP (GE Healthcare) as a running buffer.

CM5 sensor chips with an immobilized shift of more than 8000 resonance units (RU, approximately 8 μg/mm^2 of surface presentation) were used for subsequent protein binding analyses (Supporting Information Figure 3). The protein-immobilized sensor chips were rinsed with PBS buffer (10 mM, running buffer) until a stable baseline (equilibrium) was obtained, prior to the injection of the analytes. To perform binding assays, 50 μL of CD24 or sLe^x in PBS buffer at a series of concentrations was injected into the two channels (channel 2 with E-selectin and channel 1 as reference) at a flow rate of 50 μL/min, which allowed the analytes to interact with the chip surface for 1 min, followed by flowing the running buffer for 5 min. After obtaining the binding curves, the sensor chip surface was regenerated using glycin–HCl (10 mM, pH 2.5 or pH 3.0) buffer for further experiments. To confirm that the response is a result of the specific interactions, the responses (in RU) from the channel 2 were subtracted by those from the channel 1, represented as differences in responses. All sensorgrams were recorded from the start of the injection to the end of the dissociation phase and were shifted to overlay at the same baseline resonance level. All binding kinetics parameters were obtained by fitting a 1:1 binding model provided by the BIAevaluation software and represented as the mean value ± standard deviation from three independent measurements.

Cell Culture and Treatments. MCF-7 cells purchased from ATCC (Manassas, VA) were grown in DMEM media supplemented with 10% (v/v) FBS and 1% (v/v) penicillin/streptomycin in a humidified incubator at 37 °C and 5% CO$_2$. Prior to all flow chamber experiments, MCF-7 cells at a concentration of 2 × 10^5 cells/mL (5 mL) were pretreated with 240 μg/mL anti-IgG solution to prevent potential nonspecific binding.[36] To block CD24 on the MCF-7 cell surface, 100 μL of anti-CD24 (0.5 mg/mL) was added into the cell suspension, and the mixture was incubated for 15 min. The final suspensions were kept on ice throughout the subsequent cell rolling experiments. To investigate the effect of actin-filament dynamics on the cell rolling behavior of MCF-7 cells, 5 mL of the cells at 2 × 10^5 cells/mL was treated with 40 μM cytoD for 60 min, and the cells were resuspended in the supplemented DMEM media for the flow chamber experiments.[37,38]

Preparation of CD24- and sLe^x-Coated Microspheres. Recombinant human CD24 was used after dialysis (Slide-A-Lyzer mini dialysis units with 10 000 MWCO, Thermo Scientific, Rockford, IL) to remove impurities for high conjugation efficiency of the subsequent reaction with a biotin reagent. The purified, recombinant CD24 proteins were biotinylated using 10 mM EZ-Link sulfo-NHS–LC–biotin (20-fold molar excess, Thermo Scientific) for 2 h on ice, and the biotinylated CD24 proteins were repurified by dialysis using a Slide-A-Lyzer mini dialysis unit. Streptavidin-coated microspheres (104.8 μL, 1 × 10^7 beads, 9.95 μm o.d., ProActive microspheres, Bangs Laboratories, Inc., Fishers, IN) in 1% (w/v) bovine serum

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albumin (BSA) in PBS buffer (10 mM, Cellgro, with 2 mM Ca^{2+}) (BSA solution) were washed three times using a centrifuge at 10 000 rpm for 2 min. The biotinylated CD24 (100 nM) was conjugated to the microspheres in BSA solution by the spontaneous interaction between streptavidin and biotin after 1 h of incubation at room temperature. The coating of CD24 molecules on microspheres was confirmed by flow cytometry (Supporting Information Figure 4) using the same immunostaining condition for the confirmation of CD24 expression on MCF-7 cell using phycoerythrin (PE)−anti-CD24. In parallel, sLe^x-coated microspheres were also prepared under the exactly the same condition used for preparation of the CD24-coated microspheres.[39] Given that the microspheres with the same streptavidin density were used, and that the functionalization conditions were identical, the density of CD24 and sLe^x on the microspheres should be, if not identical, very similar. For parallel flow chamber experiments, CD24- or sLe^x-coated microspheres were resuspended in 10 mL of BSA solution (1×10^6 microspheres/mL). To investigate potential nonspecific interactions of microspheres themselves with E-selectin, a flow chamber experiment (described in detail below) was performed using nonfunctionalized microspheres, confirming that there is no nonspecific binding (Supporting Information Figure 2).

Surface Functionalization by Immobilization of Adhesive Proteins. A general scheme of the adhesive protein immobilization, along with the characterization of the surfaces, is described in our previous publication.[24] Briefly, 150 μL of each protein (E-selectin or anti-EpCAM) at a concentration of 5 μg/mL in PBS buffer was added on an approximately 1 cm^2 area of an epoxy-functionalized glass slide defined by a poly(dimethylsiloxane) (PDMS) gasket. After incubation at room temperature for 4 h with constant gentle shaking, the PDMS gasket was removed, and the whole slide surface was washed with PBS three times. Potential nonspecific binding of both protein-coated and uncoated regions was blocked by a final incubation with BSA solution. Experiments using the functionalized surfaces were performed immediately.

Flow Chamber Experiments. Flow chamber experiments were also performed according to our previous report.[24] The biofunctionalized glass slide, a gasket (30 mm (L) × 10 mm (W) × 0.25 mm (D), Glycotech), and a rectangular parallel plate flow chamber (Glycotech) were assembled in line under vacuum. To observe cellular interactions, MCF-7 cell suspensions with or without anti-CD24 treatment as well as CD24- or sLe^x-coated microsphere suspensions were injected into the flow chamber at various flow rates (ranging from 50 to 200 μL/min) using a syringe pump (New Era Pump Systems Inc., Farmingdale, NY). Note that, in this flow chamber, 50 μL/min of flow rate is correspondent to 0.08 dyn/cm^2 of a wall shear stress, 8 s^{-1} of a wall shear rate, and 20 μm/s of near-wall nonadherent cell velocity according to the Goldman equation.[40] All cellular responses in the flow chamber were monitored using an Olympus IX70 inverted microscope (IX 70-S1F2, Olympus America, Inc., Center Valley, PA), and images were recorded using a 10× objective and a CCD camera (QImaging Retiga 1300B, Olympus America, Inc.). Rolling velocities of cells and the functionalized microspheres on the immobilized proteins were measured, based on the images taken every second for 1 min in repetitive observations using ImageJ (NIH). Cell rolling was defined when the rolling velocities were less than 50% of the free stream velocity (e.g., slower than 10 μm/s at a flow rate of 50 μL/min). In addition to the flow-based experiments using the function-
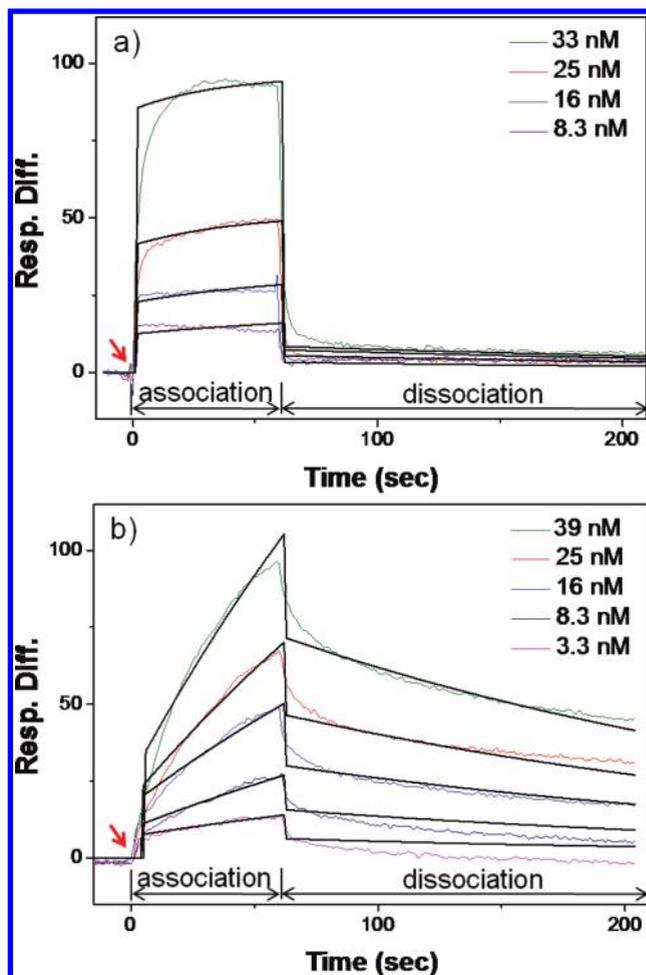

Figure 1. SPR sensorgrams showing the binding curves of (a) CD24 and (b) sLe^x with the immobilized E-selectin. The colored lines represent the raw data curves, and the solid black lines are simulated fitting curves using a 1:1 interaction model. The arrows indicate the injection time of the analytes.

Table 1. Quantitatively Measured Kinetic Parameters of Binding of CD24 and sLe^x with E-selectin Using SPR

kinetic parameters[a]	ligands	
	CD24	sLe^x
k_a (× 10^4 M^{-1} s^{-1})	37.9 ± 30.4	7.9 ± 2.6
k_d (× 10^{-3} s^{-1})	1.6 ± 1.3	5.6 ± 0.8
K_A (× 10^7 M^{-1})	31.8 ± 6.5	1.4 ± 0.3
K_D (nM)	3.4 ± 0.7	78.3 ± 15.7

[a] k_a, association rate constant; k_d, dissociation rate constant; K_A, association constant; K_D, dissociation constant. All the kinetic values were obtained by averaging three independent runs of SPR measurements, presented as averages ± standard deviations.

alized microspheres, the same number of CD24-coated microspheres (1×10^6 microspheres/mL in BSA solution) was incubated with each protein-immobilized surface for 1 min and the number of protein-immobilized microspheres per 1 mm^2 that remained on the surface was counted after flowing PBS buffer for 1 min.
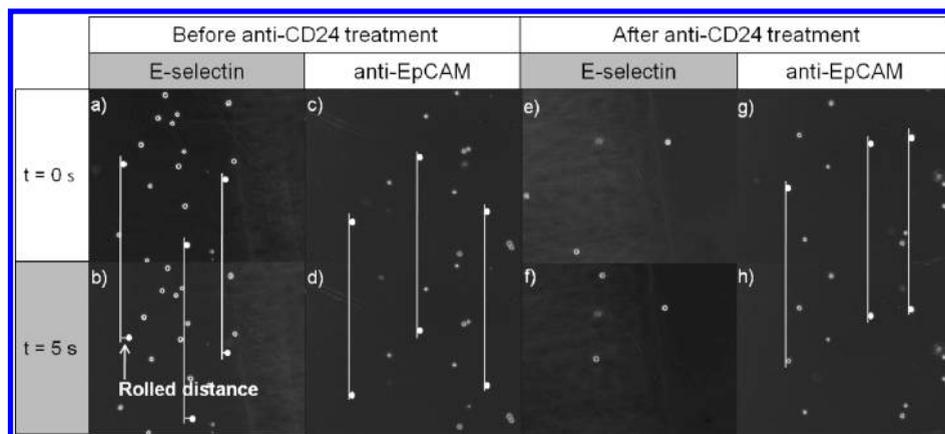
Figure 2. Time-course images of MCF-7 cells under shear stress of 0.08 dyn/cm^2 on E-selectin- and anti-EpCAM-immobilized surfaces. Untreated MCF-7 cells exhibit the rolling behavior on the E-selectin-coated surface at a velocity of $2.76 \pm 0.16\,\mu$m/s (a and b) while being stationary bound on the anti-EpCAM-coated surface (c and d). Upon treatment with anti-CD24, the interaction of MCF-7 cells with E-selectin disappeared (e and f), whereas the binding with anti-EpCAM is not affected (g and h). Note that the cells are not tracked in the images e and f due to no interaction between the anti-CD24-treated cells and the surface, resulting in fast free flow of the cells. Flow direction is from left to right.
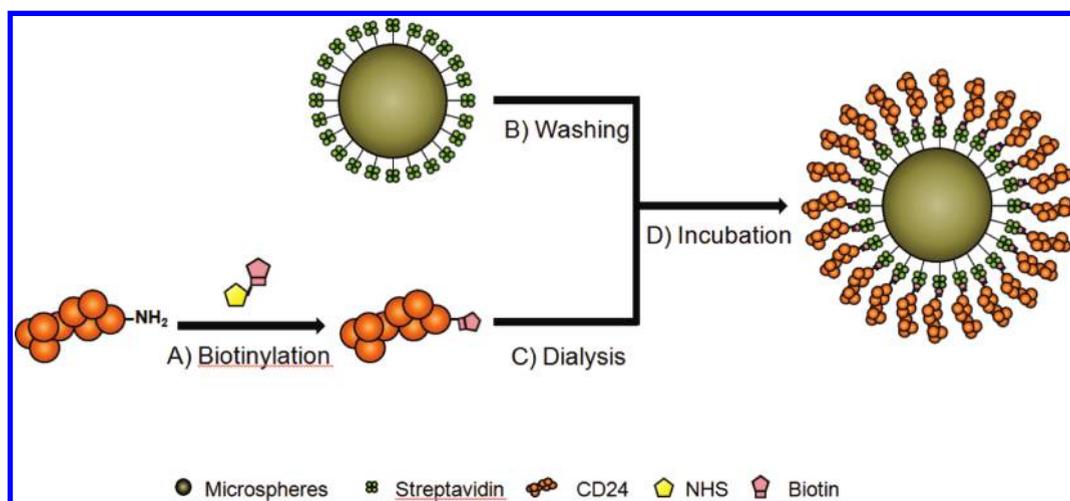

Figure 3. Preparation of CD24-coated microspheres: (a) biotinylation of recombinant human CD24, (b) prewashing of streptavidin-coated microspheres, (c) purification of the biotinylated CD24 using dialysis, and (d) incubation of the biotinylated CD24 with streptavidin-coated microspheres.

■ RESULTS AND DISCUSSION

We have previously reported MCF-7 cell rolling on immobilized E-selectin.[24] However, as indicated, it was unclear which ligand was responsible for inducing the rolling behavior since the cells do not express any known ligands for E-selectin. The purpose of this study was therefore to identify the ligand of MCF-7 cells. Given that cells that maintain a high level of CD24 expression have enhanced invasive and adhesive properties which can potentially be related to cancer metastasis,[28,32] we hypothesized that CD24 may mediate the rolling response of MCF-7 cells on E-selectin.

Direct Measurements of Binding Kinetics Using SPR. For the first time to our knowledge, the binding kinetics of recombinant CD24 with E-selectin was quantitatively measured using a direct binding assay by Biacore X. A well-known ligand for E-selectin, sLe^x, was also employed as a control. The Biacore sensorgrams illustrating the binding interactions between the E-selectin-immobilized sensor chips and CD24 or sLe^x are shown in Figure 1, and all measured kinetic parameters are listed in Table 1. Note that concentrations of the analytes (CD24 and sLe^x)

used for the SPR measurements were optimized to achieve similar levels of resonance unit (RU) (preferably lower than 100) between sensorgrams. The SPR measurements revealed that CD24 indeed bound to E-selectin. The association rate constant (k_a) and the dissociation rate constant (k_d) were $3.8 \times 10^5\,M^{-1}\,s^{-1}$ and $1.6 \times 10^{-3}\,s^{-1}$, respectively, and the dissociation constant (K_D) of CD24 was 3.4 nM. The binding parameters k_a, k_d, and K_D of sLe^x were measured to be $7.9 \times 10^4\,M^{-1}\,s^{-1}$, $5.6 \times 10^{-3}\,s^{-1}$, and 78.3 nM, respectively. Although the k_d values of CD24 and sLe^x were relatively similar, k_a of CD24 was higher than those of sLe^x by an order of magnitude. On the basis of the ratio of the directly measured binding parameters, especially the dissociation constants, we have found that the binding affinity of CD24 with E-selectin is stronger than that of sLe^x by approximately 20-fold.

Cellular Responses on Selectin- and Anti-EpCAM-Functionalized Surfaces. We then investigated how the measured binding parameters correlate with cell interactions with various proteins on surfaces under flow using a parallel plate flow chamber. As shown in our previous report, no interactions were observed between MCF-7 cells and P-selectin.[24] Although CD24
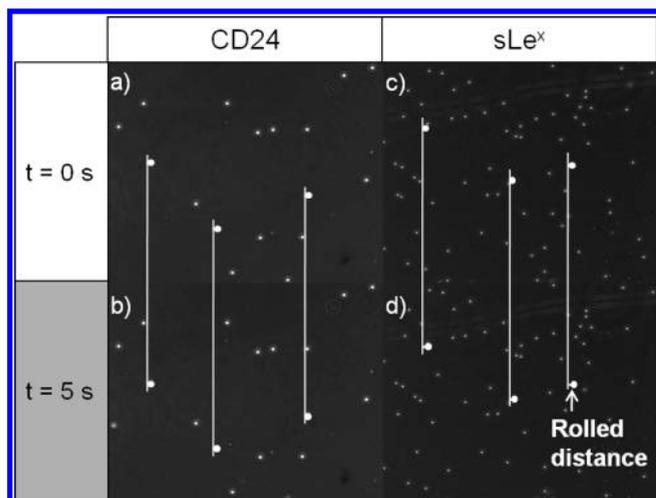
Figure 4. Time-course images of microspheres that are coated with (a and b) CD24 and (c and d) sLe^x under shear stress of 0.08 dyn/cm^2 on E-selectin-immobilized surfaces. CD24-coated microspheres are statically bound on the E-selectin-immobilized surface, but sLe^x-coated microspheres exhibit the rolling behavior on E-selectin (0.44 ± 0.02 μm/s). Flow direction is from left to right.
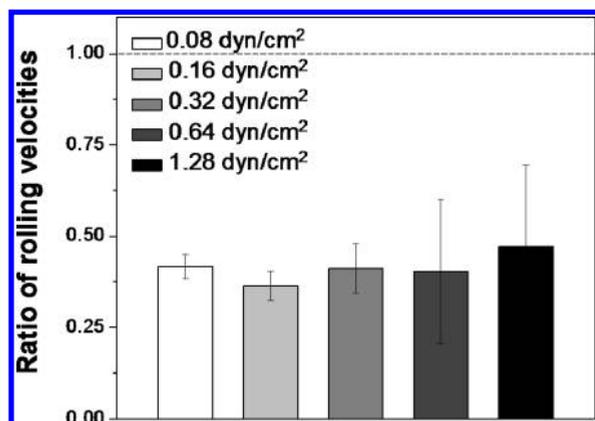

Figure 5. Ratios of rolling velocities of cytoD-treated MCF-7 cells to those of untreated cells on E-selectin-immobilized surfaces. MCF-7 cells were treated with 40 μM cytoD for 30 min. The dotted line (ratio 1) indicates the rolling velocity of the untreated MCF-7 cells. Note that the rolling velocities of the treated MCF-7 cells are significantly reduced in a shear stress independent manner. Error bars: standard error ($n = 120$).

is known as a P-selectin ligand, it requires modification by mechanical enzymes to bind to P-selectin, which requires sLe^x decoration.[26,41] This can explain the lack of interaction between sLe^x-deficient MCF-7 cells and P-selectin.[24] Figure 2 shows the surface interactions of MCF-7 cells with E-selectin and anti-EpCAM. The cell interactions with each of the individual proteins in Figure 2a–d were compared to those of the anti-CD24-treated MCF-7 cells in Figure 2e–h. Images of the cells on each surface were taken at $t = 0$ s (a randomly chosen time to begin recording during the flow experiment) and at $t = 5$ s to observe any dynamic cell response such as cell rolling. Untreated MCF-7 cells exhibited the rolling response on E-selectin and stationary adhesion on anti-EpCAM. The average rolling velocity of MCF-7 cells was 2.8 ± 0.2 μm/s on E-selectin-immobilized slides at a wall shear stress of ~0.1 dyn/cm^2. Upon anti-CD24

treatment, the rolling behavior of MCF-7 cells on E-selectin was completely interrupted (Figure 2, parts e and f), whereas the responses of MCF-7 cells on anti-EpCAM remained the same (Figure 2, parts g and h), i.e., stationary binding with anti-EpCAM. The directly measured binding kinetics between CD24 and E-selectin using Biacore, along with the ceased rolling of MCF-7 cells upon anti-CD24 treatment, confirms that CD24 is a mediator for the MCF-7 cell rolling.

Interactions of Microspheres on the Surfaces Functionalized with E-selectin. To assess if CD24 is solely responsible for binding and rolling of the MCF-7 cells, CD24-coated microspheres were prepared for a flow chamber experiment (Figure 3). Microspheres functionalized with sLe^x were also prepared according to the previous report and used as a control group.[39] Note that the streptavidin-immobilized microspheres (without CD24 or sLe^x) had no nonspecific binding with immobilized E-selectin (Supporting Information Figure 2, parts a and b). CD24- and sLe^x-coated microspheres exhibited different behaviors on the E-selectin-immobilized slides. As shown in Figure 4, CD24-coated microspheres exhibited stationary binding to E-selectin, whereas sLe^x-coated microspheres exhibited the stable rolling response on E-selectin at a velocity of 0.4 μm/s, which is consistent with the previous report.[39] The stationary adhesion of CD24 microspheres as opposed to the dynamic rolling of sLe^x microspheres can be explained by the significantly higher affinity between CD24 and E-selectin than sLe^x-based binding by ~20-fold, as measured by SPR. Additionally, in a Ca^{2+}-deficient PBS buffer, the CD24- and sLe^x-coated microspheres did not bind to E-selectin (Supporting Information Figure 2, parts c and d), indicating that the interaction of both CD24 and sLe^x with E-selectin is Ca^{2+}-dependent.

Actin-Filament Dynamics for the Cell Rolling Behavior. Our results so far still remain a question why CD24-coated microspheres bind statically (Figure 4) unlike MCF-7 cells that exhibit rolling (Figure 2) on the E-selectin-immobilized surface. This may be explained by the role of intracellular dynamics of the live cells. Given that actin-filament dynamics are known to be involved in P-selectin-mediated rolling,[42] we investigated the effect of the actin filaments on MCF-7 cell rolling. CytoD is a cell-permeable inhibitor of actin polymerization, resulting in disruption of actin network organization,[43] which alters the rolling response of neutrophils on P-selectin to the stationary adhesion.[42] MCF-7 cells were incubated with various concentrations of cytoD ranging from 10 to 40 μM for 30 and 60 min, and the rolling velocity ratios (the velocity of treated cells to that of untreated cells on E-selectin) as a function of shear stress were measured. After treating the cells with cytoD at a concentration of 40 μM for 60 min, the rolling velocity was significantly reduced to less than half of that of untreated cells (Figure 5), suggesting that actin-filament dynamics play a role in the rolling process. Intracellular dynamics are known to be involved in various cellular interactions with adhesive molecules. Actin-filament polymerization is one of the factors that affect cell morphology[37] and cellular behaviors (e.g., cell migration[44] and intercellular interactions between cancer cells and endothelial cells[45]). In our experiments, cytoD-treated MCF-7 cells exhibited an increased adhesion, i.e., the decreased rolling velocities (Figure 5). Therefore, actin-filament dynamics could be one factor that can control the intercellular interactions, which could explain the different behavior of the CD24-coated microspheres from MCF-7 cells with E-selectin. This can be further supported by the case of human colorectal carcinoma cells (HT-29), where it was reported that the adhesion

between cells and the adhesive molecules became tight under dynamic condition after disruption of actin filaments.[45]

In summary, this study reports the specific interaction between CD24 and E-selectin, which is confirmed by (i) the direct measurements of the binding kinetics of CD24 by SPR (Figure 1), (ii) the observed interruption of rolling of MCF-7 cells upon treatment with anti-CD24 (Figure 2), and (iii) the specific binding of CD24-coated microspheres on E-selectin (Figures 4 and 5). The identification of CD24 as a ligand for E-selectin will help to better understand the adhesion and invasion mechanisms of potentially metastatic cancer cells. For example, using CD24-mediated cell rolling on E-selectin-immobilized devices, CD24-positive and -negative cancer cells may be separated without staining based on different binding and rolling behaviors that are reflected by CD24 expression. Further, in a similar way to several publications,[46−48] CD24- and sLe[x]-coated microspheres may be utilized to inhibit E-selectin-mediated cell adhesion (cell migration antagonists), which can be potentially used as treatment of inflammatory diseases and metastatic cancer.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information. Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

■ ACKNOWLEDGMENT

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