Flow-Regulated Endothelial Glycocalyx Determines Metastatic Cancer Cell Activity

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Supplementary Data Legends

Figure S1: Schematic shows timeline for the cell culture experiment.

Figure S2: Computer aided simulation, showing changes in flow patterns after the introduction of a vertical step in the flow path. The DF region forms immediately after the step, which is characterized by flow detachment, eddy current, and reattachment sections. This is immediately followed by a transition region where the flow gradually changes until it become uniform. The transition region is followed by the UF region where the flow is void of any disturbances. **A**. Flow patterns generated when UF is 12 dynes/cm^2, and more details can be found in our prior publication [1]. Associated shear stress profile is also shown. This was the setting in which HUVEC were flow conditioned prior to exposure to 4T1 or MCF7 cells. **B**. Flow patterns generated when UF is 1 dyne/cm^2. These were the conditions used for co-incubation of HUVEC with circulating 4T1 or MCF7 cells. **C.** Control studies performed in the absence of ECs show that resultant CTC attachment in the 1 dyne/cm^2 condition are independent of DF versus UF. This removal of the direct impact of flow on regulation of CTC attachment will allow us to uncover the impact of the flow-regulated EC GCX on CTC-EC interactions instead, in accordance with the main purpose of our study.

Figure S3: Control static experiments for E-selectin coverage, GCX expression, endothelium integrity, and cancer cell attachment before the introduction of both DF and UF patterns to HUVEC. **A and B**. E-selectin coverage in static conditions (green is E-selectin and blue is DAPI-labeled nuclei), as it pertains to HUVEC in the presence (A) or absence (B) of TNF-α. **C and D**. Staining for GCX with WGA shows intact GCX on the surface of the HUVEC monolayer (green is WGA-labeled GCX and blue is DAPI-labeled nuclei) in the presence (C) or absence (D) of TNF-α. **E**. Phase contrast image showing healthy endothelium. Inset shows VE-cadherin labeled endothelium as further indication that endothelial layer is healthy. **F**. 4T1 breast cancer cell attachment to the endothelium.

Figure S4: Dose response experiment to establish appropriate enzyme concentration for HUVEC GCX degradation. **A**. Control sample shows intact GCX as labeled with WGA for both coverage and thickness. **B**. After treating HUVEC with 15mU/mL of Neur, we observed changes in the coverage of GCX but not the thickness. **C**. Adding 135mU/mL of Neur resulted in a significant decrease in both the coverage and thickness of GCX. **D**. After treating HUVEC with 1215mU/mL of Neur, we observed an exponential decrease in the coverage and thickness of GCX on HUVEC monolayers. **E**. Finally, by adding an increased enzyme concentration of 3645mU/ml, we noticed a total degradation of GCX on the surface of HUVEC monolayers. **F and G**. Data quantification for the coverage and thickness of GCX after dose response treatment with Neur enzyme respectively. One-way ANOVA was used to determine differences between groups, and the sample size was N=3. Statistical significance between groups is denoted as *P<0.05, ****P<0.0001, or not significant (ns).

Figure S5: A. MCF-7 cell as a singlet, versus MCF-7 cells in clustered form, versus MCF-7 cells initiating migration. **B.** Cluster of MCF-7 cells attached to HUVEC on top of HUVEC layer. **C.** MCF-7 cell appears to be underneath the HUVEC cell indicating that complete MCF-7 migration could have occurred. However, the glass slide below the HUVEC prevents the migration.

Figure S6: Diffusive *in vivo* flow cytometry (DiFC) system used to track movement of 4T1 breast cancer cells in the blood vessels of BALB/C mice. **A.** The DiFC system configuration is shown. BP: band pass filter; ND: neutral density filter; M: mirror; BS: beam splitter; FC: fiber coupler; PMT: photomultiplier tube; PA: pre-amplifier; DAQ: data acquisition board; PC: personal computer. Subscripts, x: excitation; m: emission. This image was previously published and is being used with permission [2]. **B.** Example of a peak which corresponds to a 'detected' cell on DiFC. **C**. During the measurement, fiber probes were placed

in firm contact with the surface of a mouse tail. DiFC can identify cells moving in the arteriosus (red arrows) or venous (blue arrows) direction.

Figure S7: Flow conditioned HUVEC were exposed to 4T1 or MCF7 cells in static conditions and not in 1 dyne/cm^2 flow conditions, to confirm that 1 dyne/cm^2 flow, which slightly reduces 4T1 or MCF7 residence time, does not actually impair 4T1 or MFC-7 interactions with HUVEC **A.** Number of 4T1 and MCF7 breast cancer cells attached to the DF-conditioned endothelium. The dotted line represents normalized UF data. Significant increase in the attachment of cancer cells in the DF region compared to UF region. **B**. Number of cancer cell clusters formed in the DF region. The dotted line represents normalized UF data. We observed a non-significant change in the clustering of 4T1 and MCF7 breast cancer cells to the endothelium in comparison with UF regions. This lack of clustering could be explained by the fact that we need to study a larger dataset. It could also be due to the reduced DF that we see in the 1 dyne/cm^2 setting, which probably promotes aggregation of the cancer cells. Another explanation could be that cancer cells need time in flow, which could make their surfaces more adhesive to each other. Testing these possibilities is a subject for another and outside the scope of the current project. **C**. Initiated migration of 4T1 and MCF7 breast cancer cells through the DF-conditioned endothelium. The dotted line represents normalized UF data. Compared to UF regions, we observed a significant increase in the initial migration of cancer cells through the DF region, compared to UF areas. All data "Normalized with UF". Student *t* test was used to compare DF vs. UF. Sample sizes: 4T1 attachment N=7, MCF7 attachment N=5. Significance is compared to the UF condition and denoted as *P<0.05, **P<0.01, or not significant (ns).

Figure S8: Quantification of the behavior of CTCs (multiple myeloma cells) circulating in the blood vessels within the ear of nude mice; this is further analysis of studies reported in [3, 4]. **A and B.** Raw image (A) and traced image (B) obtained from a video of blood vessels within the ear of nude mouse. Images show vessels that are branched (exposed to DF) and straight (exposed to UF). **C.** The speed of multiple myeloma CTCs traveling through branched (DF) versus straight (UF) sections of the vessel was also quantified. Graph indicates that multiple myeloma CTCs traveling in straight (UF) vessels moved at a higher speed than those traveling in branched (DF) vessels. Apparently, lack of hinderances to flow in straight (UF) vessels allows multiple myeloma CTCs to travel more freely through the vessels. **D.** Branched (DF) vessel areas versus straight (UF) vessel sections were analyzed to determine the extent to which multiple myeloma CTCs attached to branched (DF) areas of blood vessels within the ear. Quantification revealed that multiple myeloma cells preferred to attach more at branched (DF) areas of the vessel than in straight (UF) sections of the vessel. Data shown in **C and D** is not statistically significant due to lower than needed sample size, but should be considered as preliminary. **E and F.** Multiple myeloma CTC results for attachment to vessels, and speed of motion in vessels, are shown to correlate to degraded GCX that exists in ligated carotid arteries (DF) versus intact GCX that exists in non-ligated carotid arteries (UF).

Literature Cited:

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