S8:The Glycocalyx – Its Diversity

S8-1 Surface glycocalyx mediates tumor cell metastasis

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The glycocalyx layer on tumor cells has been associated with cellular functions that enable invasion and metastasis. In addition, aggressive renal carcinoma cells (SN12L1) with high metastatic potential have enhanced invasion rates compared to low metastatic (SN12C) cells in response to interstitial flow stimuli in vitro. Our previous studies suggest that heparan sulfate (HS) and hyaluronic acid (HA) in the glycocalyx play an important role in this flow mediated mechanotransduction and upregulation of invasive and metastatic potential. In our recent study, SN12L1 cells were modified to suppress HS production by knocking down its synthetic enzyme NDST1. Using modified Boyden chambers with defined interstitial flow, we showed that flow-enhanced invasion is suppressed in HS deficient cells. We also examined two prominent HSPGs on renal carcinoma cells – glypican-1 and syndecan-1 and one prominent HA receptor – CD44. We observed higher glypican-1 levels in flow dependent SN12L1 cells when compared to SN12C cells, but not syndecan-1 or CD44. Our data suggest that glypican-1 is the core protein responsible for interstitial flow sensing in metastatic cancer cells, consistent with observations in endothelial cells. To assess the ability of tumor cells to metastasize in vivo, parental or HS knockdown SN12L1 cells were injected into kidney capsules in SCID mice. Histological analysis confirmed that there was a large reduction (95%) in metastasis to distant organs by tumors formed from knockdown cells compared to control cells. The ability of these knockdown cells to invade surrounding tissue was also impaired. The substantial inhibition of metastasis and invasion upon reduction of HS suggests an active role for the tumor cell glycocalyx and glypican-1 in tumor progression.

S8-2 Visualization of heparansulfate proteoglycans in the glycocalyx and the perivascular space of 3-dimensional perfusable microvascular networks in microfluidic devices.

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The endothelial glycocalyx is known as polysaccharide coating located at the luminal interface of the endothelium. It is indispensable from normal blood vessel function. Nonetheless its role in many biological processes is still elusive. Classical approaches to study the glycocalyx in vitro employ 2-dimensional cell cultures of e.g. Human Umbilical Vein Endothelial Cells (HUVECs) under fluid motion to promote glycocalyx formation. However, these approaches do not allow to study the role of the glycocalyx in complex biological processes such as cancer cell extravasation.

Here we employed an advanced methodology that allowed growing HUVEC based 3-dimensional microvascular networks with hollow lumens in hydrogel matrices. By confining the cell culture within a microfluidic device micro-vessel openings formed that allowed perfusion of the micro-vascular network, thus mimicking physiological conditions.

Perfusion of microvascular networks with specific lectins enabled staining of the glycocalyx in live and fixed samples. Co-staining with antibodies further allowed to distinguish the endothelial glycocalyx from heparansulfate proteoglycans in the perivascular space.

The combination of this advanced 3-dimensional cell culture and the developed staining protocols allowed observing complex biological processes with time-laps life confocal microscopy. Noteworthy, the trans-endothelial migration (luminal to perivascular space) of GFP-labelled breast cancer cells at sites of local glycocalyx defects was observed.

Hence, we conclude that the here proposed methodology will allow to shed light on the complex role of the glycocalyx in many biological processes.

S8-3 Integrin-mediated adhesion is lipid bilayer and glycocalyx dependent

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Integrins are heterodimeric transmembrane proteins that facilitate that initial adhesion of cells to surfaces, nucleate the assembly of complex force-sensing focal adhesions, and are the principle ways cells communicate mechanically with the extracellular matrix. To test for bilayer sensitivity of integrin adhesion, we manipulated human aortic endothelial cell membrane properties using benzyl alcohol, which we had shown previously using idealized membranes and molecular dynamics simulations, thins liquid ordered lipid domains and subsequently measured RGD-beta1 integrin affinity using optical trap force spectroscopy and diffusion coefficients using fluorescence correlation spectroscopy (FCS). BA caused an increase of nearly 20% of RGD-integrin affinity and transitioned from single to double valency when contact time of optically trapped bead with cell was 1.5 seconds. These resullts coincided with dimerization of integrins determined using FCS. This increase in valency was abolished when cells were treated with heparinase, an enzyme that digests away surface glycocalyx. These results are consistent with a new concept of integrin-mediated adhesion in which the glycocalyx creates opportunity for nascent focal contact formation that is abolished when glycocalyx is uniformly stripped from the cell. BA caused an increase in focal-adhesion-kinase/paxillin-positive peripheral adhesions and a subsequent reduction in migration speeds. We conclude that the glycocalyx and the membrane participate cooperatively in the initial adhesion of integrins to extracellular matrix and, thus, play a synergistic role in the earliest events of mechanotransduction.

S8-4 Coupled dynamics of blood flow and endothelial glycocalyx: a large-scale molecular dynamics study

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The atomic events inside the endothelial glycocalyx layers are intimately bounded with the glycocalyx-related diseases, like in some cardiovascular or renal conditions. To reveal the dynamics of both flow and glycocalyx, an all-atom flow/glycocalyx system including 5.8 million atoms is constructed with the bulk flow velocity in the physiologically relevant ranges for the first time. The flow/glycocalyx system is simulated using a large-scale molecular dynamics method. Flow dynamics, including velocity and shear stress distributions, and corresponding statistics in the presence of the glycocalyx are presented and discussed. Meanwhile, comprehensive dynamic behaviour of the glycocalyx, particularly the dynamics of the sugar chains, is observed in response to blood flow. Based on the conformational changes of the glycocalyx constituents, potential force transmission pathways are discussed, which provides new insight into the mechanism of mechanotransduction of the glycocalyx. The molecular dynamics method used in this research provides a new angle to understand the behaviour of the glycocalyx at an atom-scale, which contributes to our knowledge of pathologies of cardiovascular and renal diseases.