Kengaku Group SEMINAR

Wed 27 Nov. 2019, 17:00-18:30

Venue:
2nd Floor Seminar Room
KUIAS/iCeMS Main Building

The Journey and the Destination: Confinement Mechanobiology

Dr.Andrew Holle

Postdoctoral Fellow and AACR Basic Research Fellow
Max Planck Institute for Medical Research; Heidelberg, Germany

17:00-17:45

Assaying ECM-conferred chemoresistance on orthogonal gradient hydrogel systems

Dr.Jennifer L. Young

Postdoctoral Fellow
Max Planck Institute for Medical Research; Heidelberg, Germany

17:45-18:30

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KUIAS

Abstract:

The Journey and the Destination: Confinement Mechanobiology

Dr.Andrew Holle

Postdoctoral Fellow and AACR Basic Research Fellow

Max Planck Institute for Medical Research; Heidelberg, Germany

The ability of cells to live under physical confinement and navigate through tight spaces has relevance in a wide variety of biological regimes, including homeostatic processes like stem cell migration or immune cell targeting and disease states like cancer or chronic inflammation. One method we use to investigate this process utilizes photolithography-generated microchannel arrays to present long, confining passages to cancer cells. We have identified distinct mesenchymal and amoeboid migration modalities in MDA-MB-231 breast cancer cells that are dependent on time, ligand composition, channel dimension, cytoskeletal activity, and intracellular signaling. The transition from mesenchymal to amoeboid modalities (MAT) was found to be a dynamic process that is driven initially by Rac1-induced protrusions and subsequently by ROCK-dependent contractility. While the cytoskeletal response to confined spaces has been studied extensively, recent research into the role of the nucleus in confined migration has implicated nuclear biophysics in a number of confinement-dependent outcomes, including enhanced metastasis and cell death. We have investigated the expression of multiple nuclear reorganization proteins, including DNA damage repair (53BP1) and nuclear envelope repair (CHMP4B), and have found that in long microchannels, cells do not display the enhanced nuclear damage observed in ‘pinch-point’ constrictions. This suggests that cellular sensing of sustained confinement allows for cytoskeletal-nuclear adaptation. One such adaptation is dynamic changes in nuclear volume that accompany cell entrance into channels and are maintained in cells post-permeation, providing a form of ‘migrational memory’. Using a panel of CRISPR-mediated knockout cell lines, we have identified the Protein Kinase D isoform PKD1 as a crucial signaling protein required for nuclear adaptation and remodeling in response to a narrow, sustained confinement. Future investigations into confined migration in diverse cellular models, including stem cells and immune cells, will likely play an important role in the development of new tissue engineering strategies.

Assaying ECM-conferred chemoresistance on orthogonal gradient hydrogel systems

Dr.Jennifer L. Young
Postdoctoral Fellow
Max Planck Institute for Medical Research; Heidelberg, Germany

Cancer cell-ECM interactions have been shown to positively influence cancer cell survival and invasion by conferring adhesion-based resistance in response to chemotherapeutic drugs, and subsequently driving metastasis into surrounding tissues. While numerous promising integrin-targeted drugs have been developed, none have been successfully implemented into clinical practice due to their inconsistent performance in effectively targeting the tumor, which could stem from the enormous molecular complexity of the tumor matrix environment. Here, we present a highly-defined platform designed to identify protective matrix properties in a robust manner, examining the effects of both ECM ligand and mechanical properties in regulating chemoresistance in breast cancer cells. We have previously developed a two-step polymerization method for creating tunable stiffness gradient polyacrylamide (PA) hydrogels with values spanning the in vivo physiological and pathological mechanical landscape, i.e. from ~ 0.1 up to 40 kPa. In order to study the influence of a wide range of ECM environments, we created dual gradient hydrogels by first synthesizing shallow, non-durotactic stiffness gradient hydrogels and subsequently fabricating a gradient of ligands orthogonal to the stiffness gradient to which breast cancer cells can attach. Ligand gradients are produced by either a gradient photomask to which proteins can be coupled to the substrate via a UV-sensitive crosslinker or by depositing a gradient of highly-ordered gold nanoparticles onto the hydrogel to which thiolated integrin subtype-specific peptidomimetics, specific to integrins $\alpha_v\beta_3$ or $\alpha_5\beta_1$, can readily attach. Linear nanoparticle gradients are created using the technique Block Copolymer Micelle Nanolithography, and can span ~ 35 to 80 nm interparticle spacing over ~ 20 mm. Using these dual gradient hydrogels, we have identified specific combinations of ligand density and substrate stiffness that affect cancer cell survival, i.e. stiffer regions with more dense ligands afford higher chemoresistance to breast cancer cells. Interestingly, we find a correlation between substrate stiffness and ligand density in promoting chemoresistance, whereby high cell survival is found in a linear regime spanning lower substrate stiffness with less dense ligands up to higher substrate stiffness with more dense ligands, indicating that cancer cells could be actively modulating ligand spacing in order to enhance their survival via the activation of pro-survival signaling cascades that originate at the cell membrane. Taken together, these data provide a better understanding of the interplay between substrate stiffness, ligand type, and ligand spacing in regulating adhesion-conferred chemoprotection in cancer cells, with the ultimate aim of establishing future targets for more effective, combinatorial cancer treatments.