Minisymposium No. 22 :

"Lymphangiogenesis in Tumors and Inflammation: From Myth to Reality "

Organizers: Dr. G. Lolas, Prof. Yihai Cao and Prof. M.A.J. Chaplain

Thursday 19th of June: 9:00 a.m.-13:00p.m.

9:00a.m. – 10:00a.m.

9:00-9:05 a.m. Welcome and Introduction by *Dr. Georgios Lolas*

9:05-9:35pm Chair: *Dr. Georgios Lolas*

Speaker: Prof. Yihai Cao (Karolinska Institute, Sweden)

Title: Mechanisms of lymphangiogenesis and metastasis

Abstract

Metastasis to regional lymph nodes is probably the most common route for cancer spread. Unlike blood-stream metastasis, the mechanism that underlies lymphatic metastasis is relatively poorly understood. We are studying the complex interplay between various growth factors and cytokines in the tumor microenvironment that contributes to tumor lymphangiogenesis and lymphatic metastasis. We have recently discovered several novel lymphangiogenic factors that significantly promote intra- and peri-tumoral lymphangiogenesis, leading to widespread lymph node metastasis. Notably, these factors could coordinately stimulate lymphangiogenesis by regulation of distinctive steps of this complex process. For example, FGF-2 and VEGF-C synergistically induce lymphangiogenesis by promoting lymphatic endothelial cell proliferation and the tip formation, respectively. Another recent example is the synergistic lymphangiogenesis activity by TNF-α and VEGF-C. These findings not only uncover the complex mechanism underlying tumor lymphangiogenesis but also provide important information for development of new cancer drugs that target tumor lymphangiogenesis.

9:35 – 10:10 p.m.

Speaker: Prof. Mihaela Skobe (Mount Sinai)

Title: Mathematical modeling predicts exponential growth of metastases in lymphatic vessels

Abstract

The lymphatic system is well recognized as an important pathway for cancer dissemination; moreover, many types of cancer can form large lesions in tumor-draining lymphatics and in the lymphatic vessels in distant organs to which they have spread. Involvement of lymphatic vessels with cancer is one of the most important negative prognostic indicators. It is not understood what impact the lymphatic microenvironment has on the growth of metastases. To explain rapid growth of metastases in the lymphatics in the absence of angiogenesis observed in mice and in humans, we have developed a 3D mathematical model of intralymphatic tumor growth. This model is based on deterministic differential equations used to describe avascular tumor growth, adapted to reflect the unique architecture of the lymphatic vasculature. Our model predicts that the cylindrical shape of the lymphatic vessel, which constrains growth of the tumor in two dimensions but allows indefinite growth along the vessel, enables higher oxygen levels throughout the tumor. The greater diffusion coefficient of oxygen in lymph further improves oxygenation of intralymphatic metastases. Improved tumor oxygenation leads to decreased tumor cell death and a rapid increase of metastatic burden in the lymphatics. Importantly, our model predicts that growth of intralymphatic metastases is

exponential. This contrasts the established view that all tumors follow Gompertzian growth kinetics. These data explain rapid growth of metastases in the absence of angiogenesis and indicate that the lymphatic niche is a favorable environment for metastatic growth.

10:10 – 10:30 Coffee break

10:35 – 11:05 Chair: *Prof. Mark Chaplain*

Speaker: Dr. Ulrike Haessler, (ETH, Zurich)

Title: Dendritic cell chemotaxis in physiological matrices in well-defined 3D gradients reveals differential response to CCL21 vs. CCL19

Directed cell migration, namely chemotaxis, is a critical cellular behavior in the immunological response towards foreign pathogens. Despite the fact that an adequate immunological response requires a precise chemotactic control, the quantitative response of dendritic cells towards defined chemokine gradients remains elusive. This is mainly due to the lack of appropriate tools, which allow establishing gradients quick enough, stable over time and still precise within a physiological 3D environment. Previously, we presented a device meeting these requirements (Haessler, U., Y. Kalinin, et al. (2009). "An agarose-based microfluidic platform with a gradient buffer for 3D chemotaxis studies." Biomed Microdevices 11(4): 827-35). Here, we show how we used this microfluidic device to gain quantitative insights into how dendritic cells respond to the two CCR7-ligands responsible for lymph-node homing, soluble CCL19 versus matrix bound CCL21.

First, we developed an integrated computational model to precisely predict matrix bound and soluble chemokine fractions. Experimental data to determine matrix binding properties were gained by fluorescently labeled CCL19 and CCL21 in conjunction with modified an ELISA assay to determine the matrix binding KD for CCL21. In a following step we fine tuned the proportion of matrix bound versus soluble CCL21 by varying matrix compositions. As readout, we observed single cell migration live under a phase contrast microscope and quantified commonly used chemokinetic and chemotactic migratory parameters.

We show that chemokinesis as indicated by the percentage of migrating cells and cellular speed in the presence of both CCR7-ligands only plays a minor role in the context of a 3D microenvironment. Furthermore, dendritic cells respond similarly to CCL19 and CCL21 at lower concentration differences, but display enhanced chemotaxis towards CCL21 at higher concentration differences. If the cells are exposed to both chemokines simultaneously, CCL21 is clearly preferred, regardless if bound to the matrix or not.

11:05 – 11:20p.m.

Speaker: Dr. Georgios Lolas (cfaed TU Dresden)

Title: The role of VEGF-C and CCR7/CCL21 in tumor Lymphangiogenesis.

Tumor survival, growth and dissemination are associated with the formation of both new blood vessels (angiogenesis) and new lymph vessels (lymphangiogenesis). Despite the longstanding recognition of the presence of the lymphatic system in several clinical studies, experimental demonstration of its role in lymphedema, lymphangiogenesis or tumor cell metastasis was until recently hindered by the lack of unique markers for the lymphatic vessels. In the current work we present an in silico model for lymphangiogenesis in tumor. The modeling focuses on key events associated with the migratory response of lymphatic endothelial cells to auto- and tumor secreted growth factors. Using parameter values based on experimental data we present numerical results, which demonstrate the process of tumor lymphangiogenesis based on VEGF-C secretion and its

correlation with peritumoral and intratumoral lymphatic presence and penetration. Furthermore, we present a 3D multi-cell simulation as a generic simplification of tumour lymphovascular invasion through the activation of CCR7/CCL21 axis in order to provide a glimpse of what may be a more active role of chemokines in lymphovascular invasion.

11:20-12:00p.m Chair: *Prof. Yihai Cao*

Speaker: Prof. Cornelia Halin (ETH, Zurich)

Title: Inflammatory lymphangiogenesis and identification of new lymphangiogenic mediators

Abstract

Lymphangiogenesis not only occurs during embryonic development and tumor growth but also accompanies many chronic inflammatory conditions, such as psoriasis, rheumatoid arthritis or inflammatory bowel disease. Increasing evidence suggests that inflammation-induced changes in the lymphatic vasculature have a profound impact on the course of inflammatory and immune responses, by modulating fluid drainage, leukocyte migration or the removal of inflammatory mediators from tissues.

Our lab employs different mouse models of acute and persistent skin inflammation to better characterize the in vivo inflammatory response of the lymphatic vasculature. Our work has revealed that lymphangiogenesis is not limited to the inflamed tissue but also results in a dramatic expansion of the lymphatic network in draining lymph nodes (LN lymphangiogenesis). In a mouse model of contact hypersensitivity (CHS)-induced skin inflammation we observed that VEGF-A produced in the inflamed skin accounted for lymphangiogenesis in the inflamed skin and also in draining LNs. To investigate how skin inflammation and changes in the lymphatic network impact lymphatic function we performed lymphatic drainage and dendritic cell migration experiments in this model. These studies revealed that drainage was compromised during both acute and persistent inflammation, indicating that an inflammation-induced, proliferative enlargement of the lymphatic network does not necessarily lead to improved lymphatic vessel function. To better study the in vivo inflammatory response of lymphatic endothelial cells (LECs) we have recently performed a microarray-based analysis of LECs isolated from resting and inflamed skin. This analysis demonstrated that the inflammatory response of LECs is highly stimulus specific and results in differential up-regulation of chemokines and adhesion molecules, depending on the inflammatory stimulus applied. Guided by our microarray data we have also started to characterize genes with previously unknown expression in LECs for their role in lymphatic vessel formation, lymphangiogenesis and lymphatic function.

12:00-12:40p.m.

Speaker: Prof. Helge Wiig (University of Bergen)

Title: Role of the extracellular matrix and lymphatics in fluid volume regulation

Abstract

Collagen and glycosaminoglycans (GAGs) constituting the extracellular matrix (ECM) may limit the space available and thus exclude macromolecules from a fraction of the interstitial fluid phase. This exclusion phenomenon is of importance for transcapillary fluid and solute exchange. We examined the range of interstitial exclusion in skin by using probes within a span of molecular weights and electrical charge, and also to tested if a change in interstitial composition occurring as a consequence of aging affected exclusion. To this end we used a novel approach, involving the exact determination of albumin concentration and mass in interstitial fluid and tissue eluate by high performance liquid chromatography and thereafter expressing the corresponding numbers relative to albumin for a set

of probe proteins assessed by quantitative proteomics. There was a highly significant positive correlation between probe Stokes-Einstein radius and fractional excluded volume, and oppositely, a statistically significant, negative correlation between probe isoelectric point and exclusion for proteins with comparable size. Aging resulted in a significant reduction in skin hydration and sulphated GAGs, and a corresponding reduced fractional excluded volume for albumin and the other macromolecular probes. We have furthermore tested whether a high salt diet results in changes in biophysical properties of the ECM including lymph drainage from the interstitium. Overall, our findings suggest that the changes in the ECM in aged skin may result in delayed adjustments of fluid perturbations and reduced ability for salt storage.

12:40 – 13:00

Speaker: Mrs Arianna Bianchi (Herriot Watt University)

Title: A Mathematical Model for Lymphangiogenesis in Wound Healing

Abstract

Several studies suggest that one possible cause of impaired wound healing is the failed or insufficient lymphangiogenesis, that is the formation of new lymphatic capillaries. Although many mathematical models have been done to describe the formation of blood capillaries (angiogenesis), very few have been proposed for this phenomenon involving the regeneration of the lymphatic vessels' network. Moreover, lymphangiogenesis is a quite different process from angiogenesis, occurring at different times and in a different manner.

Here a model of five ordinary differential equations is presented to describe the formation of lymphatic capillaries after a skin wound. The variables represent different cell densities and growth factor concentrations, and when possible the parameters are estimated from real biological data. A simulation of the model is run to compare the predicted results with the ones expected in a real-life situation.

13:00 - 13:10p.m. Closing Remarks and Discussion

13:10 – 14:00 LUNCH BREAK