Department of Medical Biochemistry and Biophysics

Studies of the Tumor-Vasculature Interface: Role of TGF-beta 1-induced Epithelial to Mesenchymal Transition

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Föreläsningssal Farmakologi, KI.

Fredagen den 15 feb, 2013, kl 09.30

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Stockholm 2013

ABSTRACT

Tumor metastasis is a complex multistep process. Among key steps that occur during metastatic spread are acquisition of tumor cell motility, intravasation of tumor cells into blood or lymphatic vessels and extravasation of tumor cells at distal sites. However, the precise mechanisms that govern these metastatic steps remain elusive. This thesis aimed to bridge the fields of tumor and vascular biology to provide new insights into the metastatic process. Results are presented indicating a role of the cytokine transforming growth factor beta (TGF-β) in activating breast cancer cells for dissemination through the lymphatic system through re-activation of a latent development process termed epithelial to mesenchymal transition (EMT). Furthermore, essential roles of the coxsackie-and adenovirus receptor (CAR) for lymph vessel development, and the sphingosine-1-phosphate receptor (S1PR1) for blood vessel stabilization are presented. We expect the findings to have impact on our understanding of the interface between tumor and vascular biology and to influence future strategies to target cancer metastasis.

In paper I, we present data identifying an essential role of CAR for normal development of lymphatic vessels in the mouse. We show that genetic deletion of the CAR gene (*Cxadr*) from E12.5 during mouse development leads to subcutaneous edema, hemorrhage and embryonic death. The lymphatic vessels in CAR-deficient mice were dilated and structurally abnormal with the presence of gaps and holes at lymphatic endothelial cell-cell junctions. In addition, blood-filled lymphatics were observed in CAR-deficient mice suggesting an incomplete separation between the blood and lymphatic vascular systems. The data demonstrate that CAR plays a crucial role in the development of lymphatic vasculature in mice through formation of lymphatic endothelial cell-cell junctions.

In paper II, we demonstrate that S1PR1 plays critical role in suppressing angiogenesis and promoting vascular stability during mouse development. S1PR1 signaling promotes cell-cell adhesion and prevents sprouting angiogenesis whereas S1PR1-deficiency leads to hypersprouting angiogenesis. These data suggest that S1PR1 signaling might protect developing blood vessels from abnormal angiogenic signals through promotion of vascular stability.

In paper III, we show that TGF-β-induced EMT promotes chemotactic migration of tumor cells through the lymphatic system by mediating crosstalk between tumor cells and lymphatic endothelial cells through the chemokine receptor 7 (CCR7) and its chemokine ligand, CCL21. Reversal of EMT process through p38 MAPK inhibition inhibited tumor cell invasion *in vitro* and migration towards the lymphatics *in vivo* suggesting that p38 MAPK inhibition may be a useful therapeutic approach to inhibit tumor cell dissemination through the lymphatic system.

In paper IV, we describe development of a novel co-culture system to study tumor cell migration and interaction with lymphatic endothelial cells within a 3-dimensional matrix component. This assay allows manipulation of tumor properties or matrix components and can be used as a platform to screen for pharmacological agents which inhibit tumor-endothelial interactions.