Microfluidics-based single-molecule live-cell imaging reveals essential role of spatiotemporal dynamics of selectin ligands on blood stem-cell rolling

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Hematopoietic stem/progenitor cell (HSPC) homing occurs via cell adhesion mediated by spatiotemporally organised ligand-receptor interactions. Although molecules and biological processes involved in this multi-step cellular interaction with endothelium have been studied extensively, molecular mechanisms of this process, in particular the nanoscale spatiotemporal behaviour of ligand-receptor interactions and their role in the cellular interaction, remain elusive. Here, we introduce a microfluidics-based super-resolution (SR) and single-molecule fluorescence imaging platform [1] and apply the method to investigate the initial essential step in the homing, tethering and rolling of HSPCs in the presence of external shear stress that is mediated by selectins, expressed on endothelium, with selectin ligands (i.e. CD44 and PSGL-1) expressed on HSPCs. Our real-time imaging revealed the formation of membrane-tethers and slings (Fig. 1), on which selectin ligands showed unique spatiotemporal dynamics that is distinct from those on the cell body. Our detailed analysis suggested that the spatial confinement of the selectin ligands together with the fast scanning of a large area by the selectin ligands increase the efficiency of selectin-ligands interaction during the rolling, resulting in slow and stable rolling of the cell on selectin.

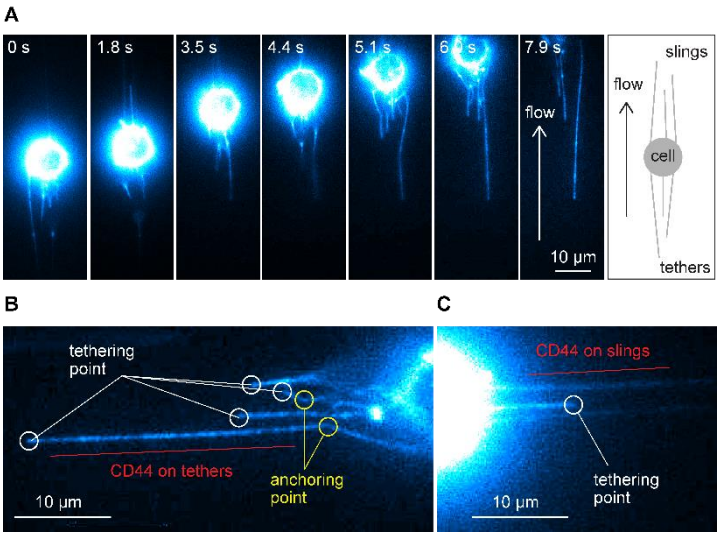

Figure 1. (A) Time-lapse fluorescence images of a selectin ligand, CD44, on a KG1a cell (working model of HSPCs) captured during the cell rolling on the surface of microfluidic chamber whose surface was coated with E-selectin in the presence of external shear stress. Formation of multiple membrane tethers (B) and slings (C) is seen clearly in the enlarged views.

[1] K. AbuZineh, L.I. Joudeh, B. Al Alwan, S.M. Hamdan, J.S. Merzaban, and S. Habuchi, "Microfluidics-based super-resolution microscopy enables nanoscopic characterization of blood stem-cell rolling" *Sci. Adv.*, **4**, eaat5304 (2018).