Studying the interactions that trap cells in circulation

Healthy blood stem cells and invasive tumor cells use similar mechanisms to exit the circulatory system and penetrate other tissues. Cell migration is a multi-step process in which a number of adhesion molecules find their partners and mediate interaction of cells in the flow to the endothelial cells lining blood vessels. Understanding precisely how these adhesion molecules capture circulating cells, particularly the connections made between the endothelial protein, E-selectin, and its ligands on circulating cells, has been difficult. One established method for studying protein-protein interactions is immunoprecipitation (IP), in which researchers use antibodies to purify a specific protein and capture other physically-associated binding partners in the process. However, IP is ineffective at detecting weak or fleeting interactions and offers limited insight into the rates at which molecules bind and separate. As an alternative we developed an IP assay based on surface plasmon resonance (SPR), a technique that can optically measure molecular interactions in real-time. SPR sensors are first prepared with antibodies that efficiently capture various known binding partners of E-selectin, and the sensors are exposed to flowing solutions of extracts from cells that express this protein followed by purified E-selectin. This approach made it possible to accurately measure the speed and durability with which E-selectin binds to two of its target proteins, known as CD44 and PSGL-1, in their native forms. Our results suggest that these ligands bound individual ‘monomeric’ molecules of E-selectin transiently, with fast on- and off-rates but bound dimeric (larger assemblies) with remarkably slow on- and off-rates. This indicates a process in which rapid but weak interactions with individual E-selectins initially capture circulating target cells and slow them sufficiently to form more durable connections with E-selectin dimers that form over time. Overall this experimental strategy now makes it possible to quantitatively dissect the molecular-scale details of the earliest stages of the migration of cells within the body with the ultimate goal of manipulating it for clinical purposes.

Publication

Quantitative Characterization of E-selectin Interaction with Native CD44 and P-selectin Glycoprotein Ligand-1 (PSGL-1) Using a Real Time Immunoprecipitation-based Binding Assay.
AbuSamra DB, Al-Kilani A, Hamdan SM, Sakashita K, Gadhoum SZ, Merzaban JS
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