System level view of molecular components shaping dendritic cell migration

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Abstract

Cell migration represents a fundamental biological activity, required for physiological processes such as organismal development, tissue homeostasis and immune reactions, but also for disease related processes such as tumor metastasis. Current knowledge suggests that most cellular motility modes are based on the components of the actin cytoskeleton, in which directed actin polymerization and its regulation by signaling cascades, and actin-branching, -crosslinkers, and –severing factors drives cellular forward locomotion. Notably, mammalian genomes encode for a large number of actin cytoskeletal components as exemplified by more than 100 described actin-binding proteins. However, the function and contribution for cellular motility of most of these proteins remains currently unknown.

Here we present experimental approaches on the unbiased genome-wide identification of actin cytoskeletal components and other molecular factors shaping the migration of dendritic cells. Dendritic cells are decisive for the initiation and regulation of adaptive immune responses. Upon encountering inflammatory signals or pathogens, dendritic cells mature and migrate from peripheral tissues to draining lymph nodes to present the peripherally acquired antigens to lymphocytes. By mimicking the physiological migration of dendritic cells in 3-dimensional collagen gels to CCL19 chemokine gradients and combining these cell migration assays with unbiased approaches, we aim to identify essential cytoskeletal components required for dendritic cell migration. Specifically, we describe the isolation and deep sequencing of total RNA (RNAseq) of dendritic cells while migrating in 3-dimensional collagen gels in CCL19 chemokine gradients. Complementary, we describe the proteomic identification of phosphorylation events induced by CCL19. By analyzing the gene expression level and the protein phosphorylation status of all known actin regulatory and cytoskeletal components, we infer on their possible importance during dendritic cell migration. Based on this knowledge, we will describe plans for candidate loss-of-function migration screens by CRISPR genome editing to investigate the functional importance of each candidate during dendritic cell migration.